

# EXHIBIT A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No.:	10/762,078	Confirmation No:	4620
Applicant(s):	Klass P. Hardeman	Group Art Unit:	1623
Filed:	January 21, 2004	Examiner:	Lawrence E. Crane
Title:	ALKYL-LINKED NUCLEOTIDE COMPOSITIONS		

Mail Stop Amendment  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**RULE 37 C.F.R. §1.132 DECLARATION  
of Dr. Steven E. Hall**

I, Dr. Steven E. Hall, do hereby declare as follows:

1. I am skilled in the art of the field of the invention described and claimed in the patent application referenced above. I received a Ph.D. in organic chemistry from the Massachusetts Institute of Technology and am currently Senior Vice President Research and Development at Serenex, Inc., the assignee of the subject patent application. I formerly was Vice President and Director of Sphinx Laboratories, Lilly Research Laboratories. I also have held senior management positions in medicinal chemistry at Bristol-Myers Squibb. A copy of my curriculum vitae is attached to this declaration.

2. I have read and understood the Office Action in the above-referenced patent application dated May 11, 2007. I also have read and understand the specification and currently pending claims of the subject application.

3. The Office Action states, in part, that the specification allegedly does not reasonably provide enablement to the method of testing encompassed by claim 31. See Office Action, at pages 2-3. For the reasons described below, I respectfully disagree.

4. Serenex, Inc., the assignee of the subject patent application, based on the teachings and disclosure of the subject patent application, currently uses embodiments of the nucleotide affinity medium disclosed and claimed in the patent application to screen for target compounds, for example, in drug discovery applications.

5. By way of example, as described in more detail herein below and as illustrated by the data presented in **Appendix A**, which is attached hereto, several known chemical compounds were screened against the purine-binding proteome of cultured human Jurkat cells using a D3 resin, which corresponds to a nucleotide solid support disclosed in Example 45 of the subject application.

6. In this example, cultured human Jurkat cells were grown to density ( $2 \times 10^9$  cells/liter), harvested, pelleted by centrifugation and flash frozen in liquid nitrogen. Buffer components and procedures used were as described previously (Graves et al. 2002, Mol. Pharmacol. 62, 1364-1372). The frozen cell pellet was thawed, homogenized in buffer, and sonicated to lyse the cells. For example, as described in Gravers et al., the cells can be lysed by mixing with an equal volume of 2X buffer and rocking for 30 min at 4 °C. A suitable buffer (1X) can be 50 mM HEPES, pH 7.5, 50 mM NaCl, 10 mM MgCl<sub>2</sub>, 0.1% Nonidet P-40, 1 mM dithiothreitol, 1 µg/mL leupeptin, 100 µg/mL pepabloc, and 1 µg/mL aprotinin, or an equivalent buffer. The cell debris was removed by centrifugation, e.g., for 1 hour at 100,000g and the resulting supernatant was mixed with D3 resin on ice with shaking. The resin was then collected in a gravity feed column, washed with buffer to remove non-specific binding, divided into smaller columns and eluted with the compounds identified in Scheme A1 of Appendix A. For elutions, all compounds can be dissolved in the buffer and adjusted to pH 7.5. The proteins eluted by each compound were then resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by silver staining.

7. As shown in Figure A1 of Appendix A, the SDS-PAGE gel shows several protein targets eluted with each compound. 5% DMSO is shown as a negative control. The primary targets for these compounds are listed in Table A1 and are highlighted with the corresponding arrows on the gel image. The additional targets identified in the assay are also listed in Table A1.

8. By way of example, as described in more detail herein below and as illustrated by the data presented in **Appendix B**, which is attached hereto, several known chemical compounds were screened against a set of three nucleotide solid supports which were identical in all respects except for the loading of the ligand; these three solid supports correspond to nucleotide solid supports disclosed in Examples 30 (L1), 45 (D3), and 46 (D4) of the subject application.

9. In this example, porcine thymus was used as the protein source. Buffer components, tissue preparation and procedures used were as described hereinabove in paragraph 6 and in Graves et al. 2002, Mol. Pharmacol. 62, 1364-1372. The cell debris was removed by centrifugation and the resulting supernatant was mixed with one of three resins (L1, D3, or D4) on ice with shaking. The resin was then collected in a gravity feed column, washed to remove non-specific binding, divided into smaller columns and eluted with the test compounds. The proteins eluted by each compound were then resolved by SDS-PAGE and visualized by silver staining.

10. As shown in Figure B1 of Appendix B, the SDS-PAGE gel shows several protein targets eluted with each of two test compounds (the entire gel images from which these data were extracted are included as Figure 2B. The number of proteins eluted by two representative compounds was highly dependent on the ligand loading level. In this example, solid-support D3, representing a 50% loading level, meaning 50% of reactive sites on the solid support are reacted with an alkyl-linked nucleotide, was superior to both the L1 (100% loading) and D4 (25%



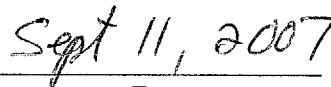
loading). This difference was observed despite the fact that the pattern of protein molecular weight (MW) standards did not differ across the three solid supports.

11. For the above reasons, based on my education and scientific experience, I believe that the specification of the subject application enables one of ordinary skill in the art to use the presently claimed nucleotide solid supports to screen a test compound as set forth in the present claims.

12. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



Dr. Steven E. Hall  
Senior Vice President Research and Development  
Serenex, Inc.  
Durham, North Carolina

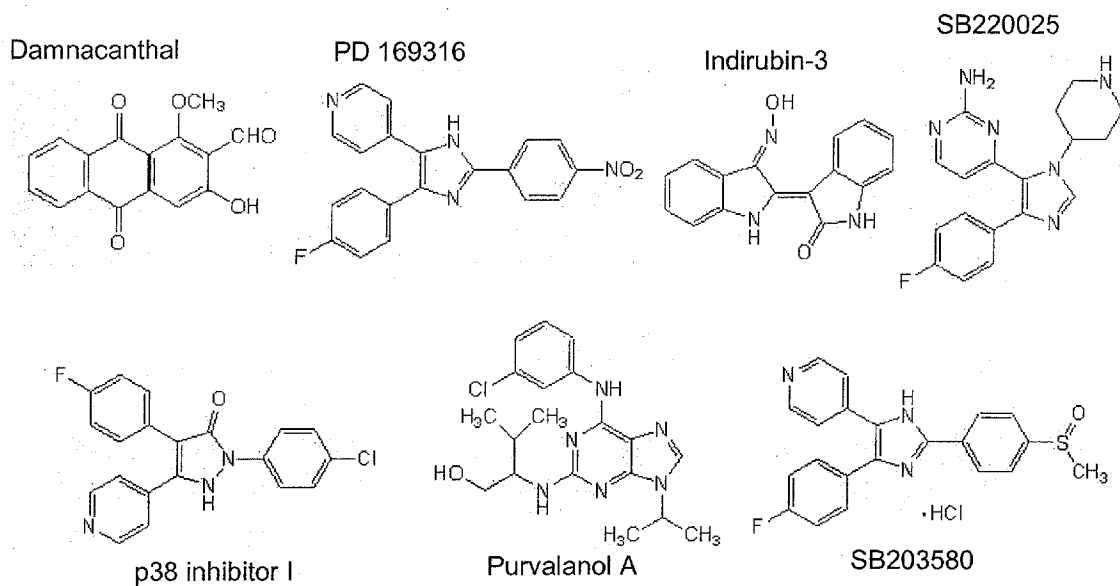


Date

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## **APPENDIX A**



Scheme A1. Representative known chemical compounds screened against the purine-binding proteome of cultured human Jurkat cells using a D3 resin.

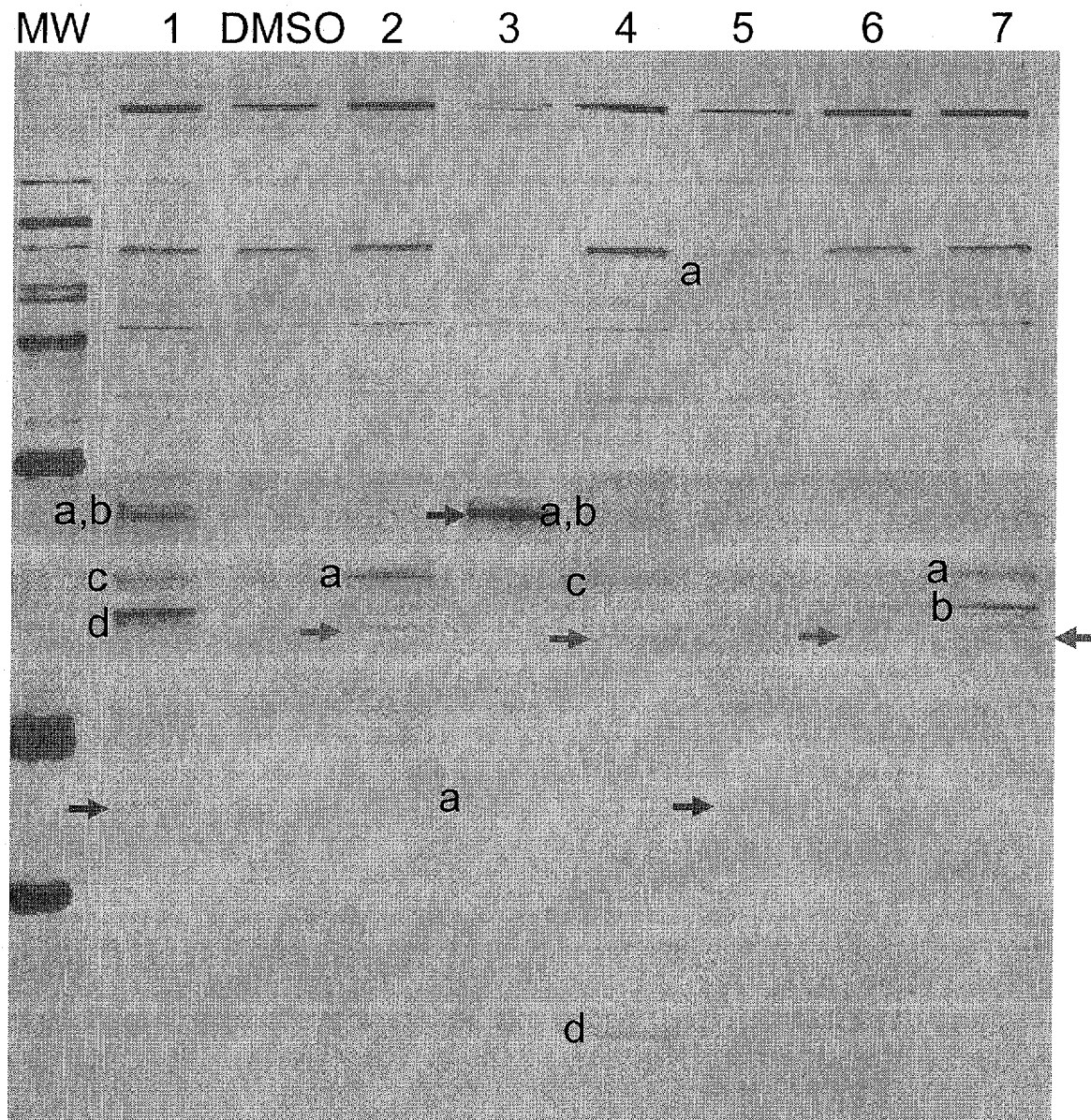


Figure A1. Representative SDS-PAGE gel showing several protein targets eluted with each compound shown in Scheme A1. Lanes labeled as MW and DMSO are the molecular weight standards and the negative control lanes (5% DMSO).

Table A1. Targets Identified in Assay Using D3 Resin.		
Compound	Expected Targets	Additional Targets
1. Purvalanol A	Cdk1/Cdk2	a. Yes b. Lck c. CSK d. MK01
2. p38 Inhibitor I	P38 MAPK	a. CSK
3. Damnacanthal	Lck	a. Cdk1/Cdk2
4. SB 203580	P38 MAPK	a. Yes b. Lck c. CSK d. DHFR
5. Indirubin-3-monoxime	Cdk1/Cdk2	a. P90 Rsk
6. PD 169316	P38 MAPK	
7. SB 220025	P38 MAPK	a. CSK b. MK01

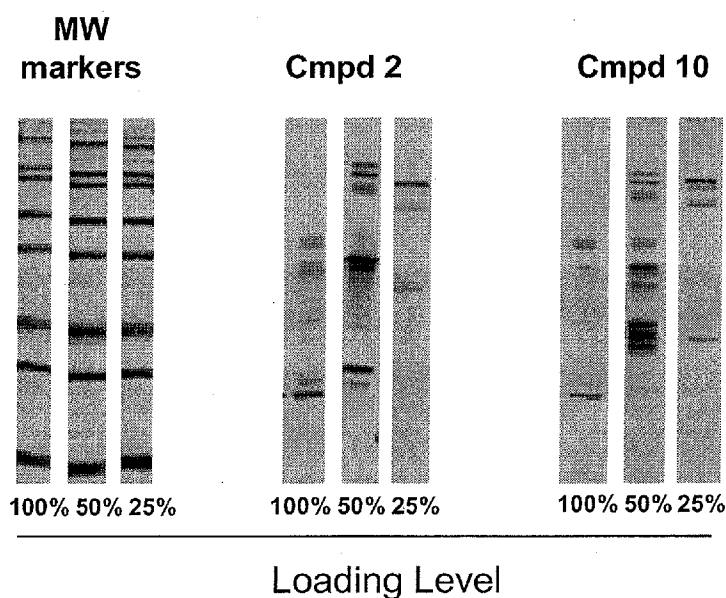
**Target Abbreviation Key for Figure A1**

<b>Abbreviation</b>	<b>Synonyms and Target Name</b>
CDK1	CDC2_HUMAN, CDC2 , Cell division control protein 2 homolog (p34 protein kinase) (Cyclin-dependent kinase 1)
CDK2	CDK2_HUMAN, CDK2 , Cell division protein kinase 2 (p33 protein kinase)
P38 MAPK	MK01_HUMAN, MAPK1, ERK2, PRKM1 , Mitogen-activated protein kinase 1 (Extracellular signal-regulated kinase 2) (ERK-2) (Mitogen-activated protein kinase 2) (MAP kinase 2) (MAPK 2) (p42-MAPK) (ERT1)
LCK	LCK_HUMAN, LCK , Proto-oncogene tyrosine-protein kinase LCK (P56-LCK) (LSK) (T cell-specific protein-tyrosine kinase)
YES	YES_HUMAN, YES1, YES , Proto-oncogene tyrosine-protein kinase YES (p61-YES) (C-YES)
CSK	CSK_HUMAN, CSK , Tyrosine-protein kinase CSK (C-SRC kinase) (Protein-tyrosine kinase CYL)
DHFR	DYR_HUMAN, DHFR , Dihydrofolate reductase
P90 RSK	KS6A1_HUMAN, RPS6KA1, RSK1 , Ribosomal protein S6 kinase alpha 1 (S6K-alpha 1) (90 kDa ribosomal protein S6 kinase 1) (p90-RSK 1) (Ribosomal S6 kinase 1) (RSK-1) (pp90RSK1)
MK01	MK01_HUMAN, MAPK1, ERK2, PRKM1 , Mitogen-activated protein kinase 1 (Extracellular signal-regulated kinase 2) (ERK-2) (Mitogen-activated protein kinase 2) (MAP kinase 2) (MAPK 2) (p42-MAPK) (ERT1)

## Appendix B

Figure 1B. Differential Elution of Proteins from Nucleotide Solid Support with Varying Ligand Loading

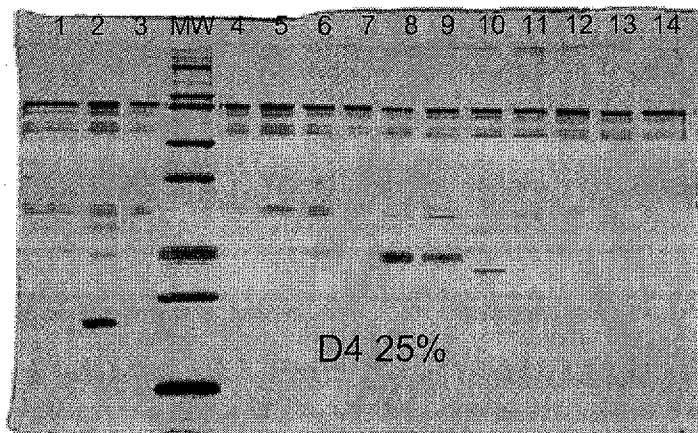
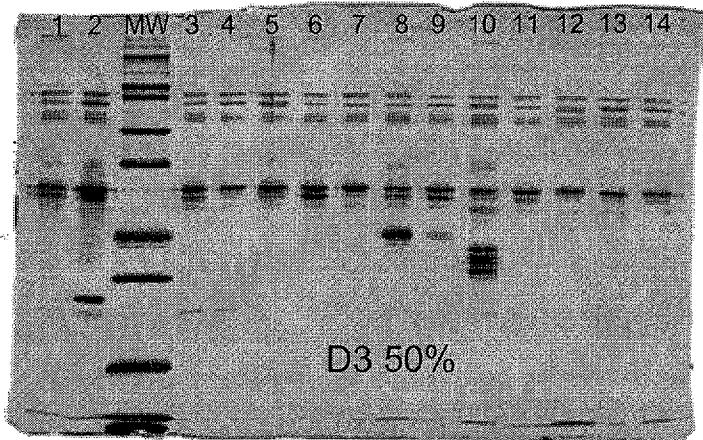
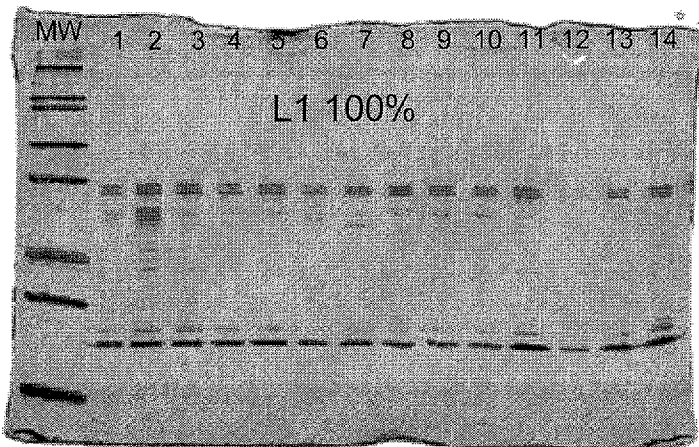
### (Controls)



### Test Compounds Used in Figure 2B

1. Bisindolylmaleimide II
2. 5-Iodotubercidin
3. Go7874
4. A3
5. Daphnetin
6. Bisindolylmaleimide I
7. H-89
8. Bisindolylmaleimide IV
9. Ro-31-8220
10. Indirubin-3'-monoxime
11. Ro-31-8425
12. Lavendustin C
13. ZM336372
14. SU6656

Figure 2B. Complete Gel Images





# Steven E. Hall, Ph.D.

## Curriculum Vitae

### Professional Experience

**Serenex, Inc., Durham, NC**

2002 – Present

**Senior Vice President, Research & Development**

- Responsible for all strategic and tactical aspects of research and technology development with a primary focus on novel drug discovery technologies to improve the conversion rate of new biological targets to drug candidates

**Eli Lilly & Company, Durham, NC**

**Site Director, Sphinx Laboratories**

2001 – 2002

- Responsible for strategic and tactical aspects of all functions at the Sphinx Laboratories site in RTP, NC
- Primary role of the site is a center of excellence for drug discovery technology, with a focus on lead generation
- Functional responsibilities include chemistry, biology, information technology, drug discovery technology, human resources, and operations
- Staff of 230; annual budget of approximately \$47M

**Director, Chemistry: Research Technologies & Lead Generation**

2000 – 2001

- Responsible for molecular diversity and lead generation chemistry research in Indianapolis, IN, RTP, NC, and Cambridge, MA
- Three major functions; natural products, corporate compound archives, and combinatorial chemistry
- Active involvement in developing overall lead generation strategy with representatives from Sphinx, Discovery Chemistry, and the Therapeutic Areas
- Staff of 155 (IN), 45 (NC), and 14 (MA)

**Sphinx Pharmaceuticals Corp., A Division of Eli Lilly & Company, Durham, NC**

**Vice President**

1997 – 2000

- Responsible for lead generation chemistry research at both RTP, NC and Cambridge, MA facilities
- Active involvement in developing and implementing overall Sphinx strategy as well as integration with Lilly Research Laboratories
- Staff of 33 (NC) and 16 (MA)

**Vice President**

1996 – 1997

- Responsible for combinatorial chemistry research (NC facility), facilities, and administration
- Short term (15 months) responsibility for biomolecular research group
- Key accomplishments include: (a) refinement of parallel synthesis process which allowed the synthesis of more than 80,000 individual compounds across more than 25 libraries, (b) one of two principals in developing combinatorial chemistry technology transfer with three Japanese pharmaceutical companies generating >\$75M, (c) effective mentoring of newly appointed biomolecular head allowed individual to assume independent role, and (d) key role in defining a revised(expanded) role for Sphinx in LRL drug discovery

- Staff of 34-50

**Vice President, Chemical Research**

1994 – 1996

- Responsible for combinatorial chemistry research (NC facility) as well as a core support group (analytical, computer support), including synthesis of novel libraries and the development of new technology
- Successfully integrated combinatorial chemistry effort with LRL Discovery
- Staff of 21.

**Sphinx Pharmaceuticals Corp., Durham, NC**

**Vice President, Chemical Research**

1993 – 1994

- Responsible for overall chemical research (NC facility) which included staff in Medicinal Chemistry, Process Chemistry, Natural Products, Combinatorial Synthesis, and Analytical Support
- Responsible for staffing as well as preparation and administration of a \$3.2M budget
- Served on committees that oversaw both the research and business activities of the Corporation
- Staff of 29

**Director, Medicinal Chemistry**

1993

- Responsible for the overall synthetic programs in the area of signal transduction mediators – primary emphasis was in the area of protein kinase C inhibitors as well as phospholipase A<sub>2</sub> (calcium-independent intracellular) inhibitors. Responsible for staffing and budget preparation
- Appointed to Sphinx-Lilly Steering Committee
- Seven direct reports and total staff of 22

**Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ**

1991 – 1993

**Associate Director**

- Responsible for the overall synthetic programs in the areas of thromboxane antagonists, blood coagulation, and potassium channel activators as well as coordinating program goals in conjunction with biological colleagues
- Developed laboratory renovation plan; funding for the prototype approved
- Division-wide responsibility for B.S./M.S. level staffing; staff of 20

**Research Group Leader – Blood Coagulation Group**

1991

- Responsible for the overall synthetic program in the design of novel antithrombotic agents
- Primary efforts involved the search for specific thrombin inhibitor

**Research Group Leader – Arachidonic Acid Metabolism Group**

1988 – 1990

- Responsible for the overall synthetic program in the development of second generation thromboxane antagonists
- Identified an extremely potent and novel class of antagonists. One member of this class is currently in clinical development
- Involved in setting up collaborations with two academic laboratories

**Senior Research Investigator – Arachidonic Acid Metabolism Group**

1987

- Involved in the development of novel thromboxane A<sub>2</sub> receptor antagonists in support of a clinical candidate

- Initiated synthetic program to identify novel thromboxane antagonists with extended duration of action
- One of two principal authors of a detailed proposal (ca. 60 pages) to initiate a program in a new therapeutic area

**Research Investigator – Arachidonic Acid Metabolism Group** 1982 – 1987

- Involved in the development of novel thromboxane A<sub>2</sub> receptor antagonists as well as cyclooxygenase inhibitors – one compound in the latter category has undergone extensive pre-clinical development
- Supervised two junior chemists (B.S. and M.S. level) in two different project areas

**Quaker Oats Company, Chemicals Division, Barrington, IL** 1976 – 1978  
**Chemist – Polymer Design Group**

- Responsible for the synthesis of new furan monomers and polymers. Also involved in process development of potential products; work included several scale-ups to pilot plant quantities
- Appointed to the Division Safety Committee in 1977.

**Education**

**Ph.D., Organic Chemistry, 1982**

**Massachusetts Institute of Technology**

**Thesis title: "Progress Towards the Total Synthesis of Chlorothricolide"**

**B.S., Chemistry (ACS-certified), 1976**

**Central Michigan University**

**Professional Activities and Awards**

- Faculty, "Accelerating Cancer Drug Development", NCI/FDA Workshop (2005)
- Session Chair, Protein Phosphorylation-Drug Discovery Summit (03/2004)
- Session Chair, Protein Kinases and Phosphatases, San Diego, CA (04/2004)
- Session Chair, Protein Phosphorylation-Drug Discovery Summit (03/2003)
- LRL Promotion review committee (1998-1999)
- Session Chair, Fifth Chemical Congress of NA, Cancun (11/1997)
- Chair, Third Random & Rational Drug Design Conference, Princeton (9/1996); San Francisco (11/1996).
- Editor, Current Medicinal Chemistry (1994-1999)
- Reviewer, J. Med. Chem.; Eur. J. Med. Chem.; Bioorg. Med. Chem. Lett.; J. Combinatorial Chem.
- BMS Promotion Review Committee, Research/Management Series(1991-1993)
- Treasurer of the Princeton ACS Fall Symposium (1984-1985).
- Co-chairman; Organic Chemistry Seminars, Squibb (1983-1986).
- Secretary-Treasurer of the Princeton ACS Fall Symposium (1983).
- Co-chair of Mini-symposium on Careers for Chemists, M.I.T.(1980)
- American Institute of Chemists Outstanding Student , CMU (1976)
- Member of the American Chemical Society, AAAS

## Publications

1. "Direct ortho-Mercuration Reactions of Azobenzene and ortho-Substituted Azobenzenes." P.V. Roling, D.D. Kirt, J. L. Dill, S. E. Hall, C. Hallstrom, *J. Organomet. Chem.* 1976 116, 39.
2. "Stereoselective Syntheses of Substituted Methyl (Z,E,E)-Deca-2, 7, 9-trienoates and Substituted Methyl (Z,E,E)-Undeca-2, 8, 10-trienoates." W. R. Roush, H. R. Gillis, S. E. Hall, *Tetrahedron Letters* 1980 21, 1023.
3. "Studies on the Total Synthesis of Chlorothricolide: Stereochemical Aspects of the Intramolecular Diels-Alder Reactions of Methyl Undeca-2,8,10-trienoates." W. R. Roush, S. E. Hall, *J. Am. Chem. Soc.* 1981 103, 5200.
4. "Synthesis of the Bottom-Half of Chlorothricolide." S. E. Hall, W. R. Roush, *J. Org. Chem.* 1982 47, 4611.
5. "Inhibition of Prostaglandin Biosynthesis by SQ 28,852, A 7-Oxabicyclo[2.2.1]heptane Analog." D. N. Harris, M. B. Phillips, I. M. Michel, H. J. Goldenberg, T. E. Steinbacher, M. L. Ogletree, S. E. Hall *Prostaglandins* 1986 31, 651.
6. "9,11-Epoxy-9-Homo-14-Oxaprosta-5-enoic acid Derivatives - Novel Inhibitors of Fatty Acid Cyclooxygenase." S. E. Hall, W.-C. Han, M. F. Haslanger, D. N. Harris, M. L. Ogletree *J. Med. Chem.* 1986 29, 2335.
7. "Characterization of (5,6-<sup>3</sup>H)-SQ 29,548 as a High Affinity Radioligand, Binding to Thromboxane A<sub>2</sub>/Prostaglandin H<sub>2</sub> Receptors in Human Platelets." A. Hedberg, S. E. Hall, M. L. Ogletree, D. N. Harris, E. C. Lui; *J. Pharm. Exp. Ther.* 1988 245, 786.
8. "7-Oxabicycloheptane Analogs: Modulators of the Arachidonate Cascade." D. N. Harris, S. E. Hall, A. Hedberg, M. L. Ogletree; *Drugs of the Future* 1988 13, 153.
9. "9,11-Epoxy-9-Homo-14-Thiaprosta-5-enoic acid Derivatives - Potent Thromboxane A<sub>2</sub> Antagonists." S. E. Hall, W.-C. Han, D. N. Harris, A. Hedberg, M. L. Ogletree *J. Med. Chem.* 1989 32, 974.
10. "9,11-Epoxy-9-Homo-Prosta-5-enoic acid Derivatives as Thromboxane A<sub>2</sub> Receptor Antagonists." J. Das, S. E. Hall, M. Nakane, J. Reid, D. Garber, V. C. Truc, M. F. Haslanger, D. N. Harris, M. L. Ogletree *J. Med. Chem.* 1990 33, 1741.
11. "7-Oxabicyclo[2.2.1]heptyl Carboxylic Acids as Thromboxane A<sub>2</sub> Antagonists: Aza  $\omega$ -Chain Analogs." M. Nakane, J. A. Reid, J. Das, V. C. Truc, M. F. Haslanger, D. Garber, D. N. Harris, A. Hedberg, M. L. Ogletree, S. E. Hall *J. Med. Chem.* 1990 33, 2465.
12. "Interphenylene 7-Oxabicyclo[2.2.1]heptane Thromboxane A<sub>2</sub> Antagonists. Semicarbazone  $\omega$ -chains." R. N. Misra, B. R. Brown, W.-C. Han, D. N. Harris, A. Hedberg, M. L. Webb, S. E. Hall *J. Med. Chem.* 1991 34, 2882.

13. "Thromboxane Receptor Antagonist BMS 180,291: A New PreClinical Lead." R. N. Misra, B. R. Brown, P. M. Sher, M. M. Patel, W.-C. Han, S. E. Hall, D. M. Floyd, P. W. Sprague, D. N. Harris, A. Hedberg, M. L. Ogletree, W. A. Schumacher, M. L. Webb, G. C. DiDonato, R. A. Morrison, R. E. Ridgewell, R. E. White; *Bioorg. Med. Chem. Lett.*, 1992, 2, 73.
14. "Purification of the Human Blood Platelet Thromboxane A<sub>2</sub>/Prostaglandin H<sub>2</sub> Receptor Protein." S.-O. Fim, C. T. Lim, S. C.-T. Lam, S. E. Hall, D. Komiotis, D. L. Venton, G. C. Le Breton *Biochemical Pharmacology* 1992, 43, 313.
15. "Pharmacological Characterization of Potent, Long Acting Thromboxane Receptor Antagonists, SQ 33,261 and SQ 33,552." D. N. Harris, I. M. Michel, H. J. Goldenberg, K. S. Hartl, G. T. Allen, T. E. Steinbacher, W. A. Schumacher, W.-C. Han, S. E. Hall, D. M. Floyd, M. L. Ogletree *J. Pharmacol. Exp. Ther.* 1992, 261, 131.
16. "Synthesis of Pyrrolidine Oxazoles as Thromboxane A<sub>2</sub>/Endoperoxide Receptor Antagonists." S. E. Hall, W.-C. Han, D. N. Harris, H. J. Goldenberg, I. M. Michel, H. Monshizadegan, M. L. Webb, *Bioorg. Med. Chem. Lett.*, 1993, 3, 1263.
17. "Syntheses of Cyclopentane, Cyclohexene, and Olefin Oxazoles as Thromboxane A<sub>2</sub>/Endoperoxide Receptor Antagonists." J. Das, S. E. Hall, J. A. Reid, W.-C. Han, D. N. Harris, H. J. Goldenberg, I. M. Michel, H. Monshizadegan, M. L. Webb, *Bioorg. Med. Chem. Lett.*, 1993, 3, 1267.
18. "Interphenylene 7-Oxabicyclo[2.2.1]heptane Oxazoles. Highly Potent, Selective, and Long-Acting Thromboxane A<sub>2</sub> Receptor Antagonists." R. N. Misra, B. R. Brown, P. M. Sher, M. M. Patel, S. E. Hall, W.-C. Han, J. C. Barrish, O. Kocy, D. N. Harris, H. J. Goldenberg, I. M. Michel, W. A. Schumacher, H. Monshizadegan, M. L. Webb, M. L. Ogletree; *J. Med. Chem.*, 1993, 36, 1401.
19. "Novel PKC Inhibitory Analogs of Balanol with Replacement of the Ester Functionality." G. E. Jadgman, Jr., J. M. Defauw, Y.-S. Lai, H. M. Crane, S. E. Hall, J. A. Buben, H. Hu, P. A. Gosnell. *Bioorg. Med. Chem. Lett.*, 1995, 5, 2015.
20. "Synthesis of Bisindolylmaleimid Macrocycles." M. R. Jirousek, J. R. Gillig, D. A. Neel, C. J. Rito, D. O'Bannon, W. F. Heath, J. H. McDonald III, M. M. Faul, L. L. Winnerowski, A. Melikian-Badalian, M. Baevsky, L. M. Ballas, S. E. Hall. *Bioorg. Med. Chem. Lett.*, 1995, 5, 2093.
21. "Ring Size Effect in the PKC Inhibitory Activities of Perhydroazepine Analogs of Balanol." Y.-S. Lai, D. S. Menaldino, J. B. Nichols, G. E. Jadgman, Jr., F. Mylott, J. Gillespie, S. E. Hall. *Bioorg. Med. Chem. Lett.*, 1995, 5, 2151.
22. "(S)-13-[(dimethylamino) methyl]-10, 11, 14, 15-tetrahydro-4, 9:16, 21-dimetheno-1H, 13H-dibenzo[e,k]pyrrolo[3,4-h] [1,4,13] oxadiazacyclohexadecene-1, 3(2H)-dione (LY333531) and related analogues: isozyme selective inhibitors of protein kinase C beta." M. R. Jirousek, J. R. Gillig, W. F. Heath, C. M. Johnston, J. H. McDonald III, D. M. Neel, C. J. Rito, L. E. Stramm, A. Melikian-Badalian, M. Baevsky, L. M. Ballas, S. E. Hall, M. M. Faul, L. L. Winnerowski, *J. Med. Chem.* 1996, 39, 2664.
23. "Synthesis and Protein Kinase C Inhibitory Activities of Indane Analogs of Balanol." H. Hu, S. P. Hollinshead, S. E. Hall, K. Kalter, L. M. Ballas, *Bioorg. Med. Chem. Lett.*, 1996, 6, 973.

Michael P., Mendoza, Jose S., Murphy, Marcia M., Wilson, Joseph W., Ballas, Lawrence M., Carter, Kiyomi, Darges, James W., Davis, Jefferson E., Hubbard, Frederick R., Stamper, Mark L. *J. Med. Chem.* 2002, 45(12), 2624-2643.

35. "Molecular design and structure – activity relationships leading to the potent, selective, and orally active thrombin active site inhibitor BMS-189664." Das, J.; Kimball, SD; Hall, SE; Han, WC; Iwanowicz, E.; Lin, J; Moquin, RV; Reid JA; Sack, JS; Malley, MF; Chang, CY; Chong, S; Wang-Iverson, DB; Roberts, DG; Seiler, SM; Schumacher, WA; Ogletree, ML. *Bioorg Med. Chem. Lett.*, Jan. 7 2002; 12(1): 45-9.

#### **Invited Lectures/Publications**

1. *Industrial Chemistry Seminar Series*, University of Texas at Austin, April 1987.
2. *Squibb Science & Technology/Scientific Seminar Series*, November 1988.
3. *Squibb Analytical R & D Seminar Series*, February 1989.
4. *Symposium on Thromboxane Modulation, Division of Medicinal Chemistry*, 197th ACS meeting, Dallas, Texas, April 1989.
5. *Bristol-Myers Squibb Analytical R & D Seminar Series*, June 1991.
6. "Thromboxane A<sub>2</sub> Receptor Antagonists." S. E. Hall, Medicinal Research Reviews, 11, 1991 (503-579).
7. "Platelet Aggregation Inhibitors." J. Das, S. E. Hall, Current Cardiovascular Patents, 1991 (221-240).
8. *Symposium on Protein Kinase C*, NE Section American Chemical Society, December 1993.
9. "A Decade of 7-Oxabicycloheptanes." S. E. Hall, Current Topics in Medicinal Chemistry, 1993, 1, 223.
10. *Combinatorial Chemistry*. Drew University Residential School in Medicinal Chemistry, June 1996.
11. *Universal Libraries in Lead Generation*. Third Random & Rational Drug Design Conference, Princeton, September 17, 1996; San Francisco, November 1996.
12. *Plenary Lecture: "Serial Processes in Drug Discovery: Old Habits Are Hard to Break."* Japanese Combinatorial Chemistry Club, Osaka, Japan. January, 1997.
13. *Combinatorial Chemistry in Drug Discovery*. Drew University Residential School in Medicinal Chemistry, June 1997.
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55. D. W. Beight, T. J. Craft, J. B. Franciskovich, T. Goodson, Jr., S. E. Hall, D. K. Herron, S. Joseph, V. J. Klimkowski, J. A. Kyle, J. J. Masters, J. S. Sawyer, R. T. Shuman, G. F. Smith, A. L. Tebbe, J. M. Tinsley, M. R. Wiley, Y. K. Yee. *Antithrombotic Amides*. U.S. Patent 6,710,057 (3/23/04)
56. D. W. Beight, T. J. Craft, J. B. Franciskovich, T. Goodson, Jr., S. E. Hall, D. K. Herron, S. Joseph, V. J. Klimkowski, J. A. Kyle, J. J. Masters, D. Mendel, G. Milot, M. M. Pineiro-Nunez, G. F. Smith, A. L. Tebbe, J. M. Tinsley, M. R. Wiley, Y. K. Yee. *Antithrombotic Amides*. U.S. Patent 6,716,839 (4/6/04)
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# APPENDIX

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

Appl. No.: 10/762,078 Confirmation No.: 4620  
Applicant(s): Hardeman *et al.*  
Filed: January 21, 2004  
Art Unit: 1623  
Examiner: Crane, Lawrence E.  
Title: ALKYL-LINKED NUCLEOTIDE COMPOSITIONS

Docket No.: 047446/273072  
Customer No.: 00826

Mail Stop AF  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**STATEMENT UNDER 37 C.F.R. §1.125(b)  
REGARDING SUBSTITUTE SPECIFICATION**

I hereby state that the Substitute Specification filed concurrently herewith is in compliance with 37 C.F.R. §1.125. The Substitute Specification incorporates the originally filed specification and drawings, and contains no new matter. In accordance with 37 C.F.R. §1.125(b)(2), a marked-up version of the Substitute Specification showing all of the changes (including the matter being deleted from the specification) is also enclosed.

The Specification has been amended throughout to replace the term "alkyl-linked nucleotide composition(s)" with the term "alkyl-linked nucleotide non-homogeneous solid support(s)." No new matter has been added by way of amendment.

Respectfully submitted,

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## ALKYL-LINKED NUCLEOTIDE COMPOSITIONS

### 5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Applications 60/453,697, filed January 22, 2003, and 60/532,134, filed December 23, 2003, each of which are hereby incorporated in its entirety by reference herein.

### 10 FIELD OF THE INVENTION

The present invention relates to nucleotide affinity media and methods for their use.

### BACKGROUND OF THE INVENTION

15 Previous methods of preparing ligands, such as nucleotides, for use in affinity chromatography have typically coupled the nucleotide to a solid matrix through the N6 amino group on the purine ring, or via a hydroxyl group of the ribose moiety. However, these ligands are not always effective ligands for affinity chromatography, usually because of steric hindrance or the orientation of the ligand on the solid matrix. The  
20 studies of the molecular structures of some nucleotide binding proteins, such as kinases, support these findings.

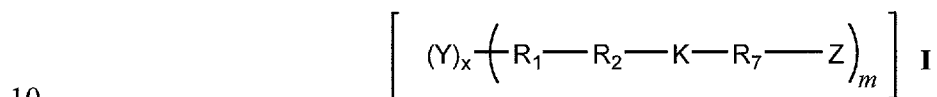
Recently, in an alternative method, the nucleotide, adenosine triphosphate (ATP), was coupled to an affinity resin indirectly through the gamma-phosphate group of ATP via an aminophenyl moiety. Linking ATP to a resin via an aminophenyl group attached  
25 to the gamma-phosphate of ATP has advantages over earlier nucleotide affinity media.

However, a need still exists to develop a still more efficient method for synthesis of nucleotide affinity media that are suitable for use in affinity chromatography and screening methods.

## SUMMARY OF THE INVENTION

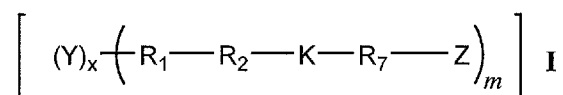
The present invention is directed to alkyl-linked nucleotide ~~compositions~~ non-homogeneous solid supports, which include alkyl-linked nucleotide affinity media.

In one embodiment, an alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support has the general formula:



such that: Y is a solid support, a tag, or a protective group; x is either 0 or 1; R<sub>1</sub> is a covalent bond between Y and R<sub>2</sub>, or R<sub>1</sub> is an acyl group, a substituted or a non-substituted group and the substituted or non-substituted group is selected from an alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl, or a heteroaryl group, or a combination of these groups; R<sub>2</sub> is a substituted or a non-substituted group, and the substituted or non-substituted group is selected from an alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, or a heteroaryl group, or a combination of these groups; K is a heteroatom; R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate and n is at least one or R<sub>7</sub> is a phosphate group mimic, Z is a nucleoside or nucleoside derivative; and m is at least one.

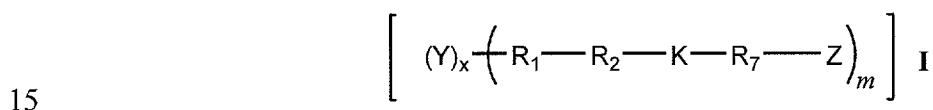
Also included in the invention is a method to synthesize an alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support comprising an alkyl-linked nucleotide affinity medium, said alkyl-linked nucleotide affinity medium having a general formula:



comprising the general steps of (a) coupling at least one linker to a solid support or tag in a suitable coupling buffer, wherein the linker is R<sub>2</sub>, or a combination of R<sub>1</sub> and R<sub>2</sub>; (b) end-capping reactive sites remaining on the solid support or tag after the coupling step; and (c) reacting a terminal phosphate or thiophosphate group of a nucleotide with the linker coupled to the solid support or tag, wherein Y is a solid support or a tag; x = 1; R<sub>1</sub> is a covalent bond between Y and R<sub>2</sub>, or R<sub>1</sub> is an acyl group, a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted

5 heteroaryl group, or a combination thereof; R<sub>2</sub> is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof; K is a heteroatom; R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate and n is at least one or R<sub>7</sub> is a phosphate group mimic, Z is a nucleoside or nucleoside derivative; and m is at least one.

Also included in the invention is a method for screening compounds. For example, the method comprises the steps of (a) contacting a proteome with a nucleotide affinity medium comprising a general formula:



wherein Y is a solid support or a tag; x = 1; R<sub>1</sub> is a covalent bond between Y and R<sub>2</sub>, or R<sub>1</sub> is an acyl group, a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof; R<sub>2</sub> is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof; K is a heteroatom; R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate and n is at least one or R<sub>7</sub> is a phosphate group mimic, Z is a nucleoside or nucleoside derivative; and m is at least one; (b) washing the nucleotide affinity medium with a buffer, whereby non-specifically bound components of the proteome are eluted from the nucleotide affinity medium and specific components of the proteome remain bound to the nucleotide affinity medium; (c) contacting the nucleotide affinity medium bound to specific components of the proteome with at least one test compound; (d) eluting from the nucleotide affinity medium components of the proteome that are specifically displaced by the test compound; and (e) identifying the components of the proteome that are specifically displaced by the test compound from the nucleotide affinity medium.

5           The alkyl-linked nucleotide ~~compositions~~ non-homogeneous solid supports and  
 alkyl-linked nucleotide affinity media as described herein are particularly useful, for  
 example, as affinity ligands in affinity chromatography methods, for the screening of  
 proteomes or combinatorial libraries, and for the purification of compounds such as, for  
 example, proteins. Furthermore, the invention includes a more efficient method for the  
 10 synthesis of such alkyl-linked nucleotides and nucleotide affinity media than has been  
 previously accomplished.

### BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic of the synthesis of a compound Intermediate IA using  
 15 cyanogen bromide-activated beaded agarose and a linker.

Fig. 2 is a schematic of the synthesis of a compound Intermediate IB using 1,1'-  
 carbonyldiimidazole (CDI)-activated beaded agarose and a linker.

Fig. 3 is a schematic of the synthesis of a compound Intermediate II.

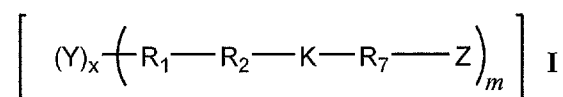
Fig. 4 is a schematic of the synthesis of a  $\gamma$ -phosphate-linked ATP using  
 20 Intermediates IA and II as reaction components.

Fig. 5 is a schematic of the synthesis of a  $\gamma$ -phosphate-linked ATP using  
 Intermediates IB and II as reaction components.

### DETAILED DESCRIPTION OF THE INVENTION

25           The features and other details of the invention, either as steps of the invention or  
 as combinations of parts of the invention, will now be more particularly described and  
 pointed out in the claims. It will be understood that the particular embodiments of the  
 invention are shown by way of illustration and not as limitations of the invention. The  
 principle features of this invention can be employed in various embodiments without  
 30 departing from the scope of the invention.

The present invention relates to an alkyl-linked nucleotide ~~composition~~ non-  
 homogeneous solid support comprising the general formula:



5 wherein Y is a solid support, a tag, or a protective group;  $x = 0$  or  $1$ ;  $R_1$  is a covalent bond between Y and  $R_2$ , or  $R_1$  is an acyl group, a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a  
10 combination thereof;  $R_2$  is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof; K is a heteroatom;  $R_7$  is  $(P)_n$  where P is a phosphate or thiophosphate and n is at least one or  $R_7$  is a phosphate group mimic, Z is a  
15 nucleoside or nucleoside derivative; and m is at least one.

Such substituted and non-substituted alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl, heteroaryl groups, and combinations of same, can be linear or branched chains, as will be understood by one of skill in the art.

A "heteroaryl," as that term is used herein, is an aryl group that includes at least  
20 one aromatic ring structure in which one or more of the atoms of at least one of the aromatic rings is an element other than carbon, for example, sulfur, nitrogen or oxygen. Examples of heteroaromatic compounds include pyridine, pyrimidine, oxazole, quinoline, thiophene and furan.

A heteroatom (K) is preferably a nitrogen atom (N), an oxygen atom (O), or a  
25 sulfur atom (S). Preferably, the heteroatom (K) is a nitrogen atom.

An alkyl-linked nucleotide is also referred to herein as a ligand or an affinity ligand.

An alkyl-linked nucleotide bound to a solid support or a tag, such that the solid support or tag is suitable for the separation of the alkyl-linked nucleotide, and optionally,  
30 compounds (such as proteins, for example) bound to the alkyl-linked nucleotide, from unbound compounds, is also referred to herein as a "nucleotide affinity medium or media", or as an "alkyl-linked nucleotide affinity medium or media".

A solid support (Y) can be any suitable support, such as a resin, or a particulate material, such as a bead, or a particle. Alternatively, a solid support can be a continuous  
35 solid surface, such as a plate, chip, well, channel, column or a tube. The material of a

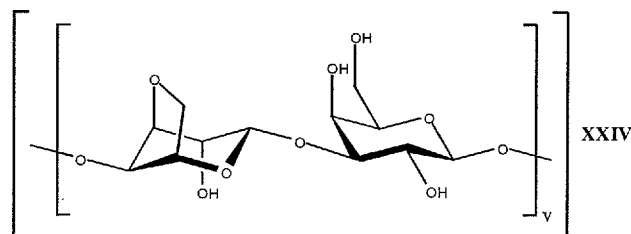
5 solid support will be of any suitable substance, compound or polymer, as will be appreciated by one of skill in the art. Examples include, without limitation, acrylamide, agarose, methacrylate polymers, methacrylate copolymers, cellulose, nylon, silica, glass, ceramic, a magnetized particle or surface, nitrocellulose, polystyrene, thermoresponsive polymers (see, for example, Lee, et al. (1996) *Journal of Applied Polymer Science* 62: 301-311; Yoshida, et.al. (1996) *Macromolecules* 29:8987-89; and Osada and Khokhlov, eds. (2001) *Polymer Gels and Networks* (Marcel Dekker, New York); each of which is  
10 herein incorporated by reference in its entirety), and derivatives thereof.

In one embodiment, the solid support (Y) is a SEPHACRYL™ resin. SEPHACRYL™ is an acrylamide derivative, produced by polymerizing allyl dextran  
15 with the cross-linking monomer N,N'-methylene-bisacrylamide.

In another embodiment, the solid support (Y) is a TOYOPEARL® resin. TOYOPEARL® is a methacrylate derivative, produced by the co-polymerization of glycidyl methacrylate, pentaerythritol dimethylmethacrylate and polyethylene glycol.

In one embodiment, the solid support (Y) is a beaded agarose. An example of a  
20 suitable beaded agarose is SEPHAROSE™ beaded agarose. Beaded agarose, such as SEPHAROSE™ beaded agarose, can be a cross-linked preparation, such as will be appreciated by one of skill in the art. Cross-linked preparations are generally recognized to have good chemical and physical stability properties. The choice of a suitable solid support will be apparent to one of skill in the art from the known characteristics of a solid  
25 support and the method of use of that solid support.

Agarose is a linear polymer with a basic structure as follows:



wherein v is at least one. Variations of this basic structure of agarose will be recognized by one of skill in the art. The preparation and use of solid supports, such as agarose, are  
30 well known in the art (see, for example, Cuatrecasas and Anfinsen, "Affinity Chromatography" in Ann. Rev. Biochem. Snell et al., eds. (CA: Annual Reviews Inc.),

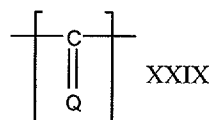
5 40: 259-278 (1971), the teachings of which are incorporated herein by reference in their entirety).

As used herein, a tag is an agent that provides for the specific detection or capture of the alkyl-linked nucleotide. For example, and without limitation, a suitable tag is biotin, avidin, streptavidin, a hapten, a fluorophore or a chromophore. Detection or  
10 capture of the alkyl-linked nucleotide employs techniques that are standard in the art. For example, a biotin-tagged alkyl-linked nucleotide can be captured using avidin, streptavidin or related avidin derivatives. The biotin-avidin interaction is highly specific. An avidin-conjugated agent used to detect or capture a biotin-tagged alkyl-linked nucleotide can be soluble (for example, an antibody), or a particulate material (for  
15 example, beaded agarose or a magnetized particle), or a continuous surface (for example, a plate or well coated with avidin). Detection and capture of a hapten-tagged alkyl-linked nucleotide can be achieved, for example, using hapten-specific antibodies. Visual detection methods for fluorophore or chromophore-tagged alkyl-linked nucleotides are readily understood by one of skill in the art.

20 Protective groups are well-known and standard in the art. The selection of a protective group will be dependent upon the properties of the reactive group, the conditions in which the compound is to be used and the function that is desired. These are readily understood by those of skill in the art (see, generally, "Protective Groups in Organic Synthesis" Greene and Wuts, eds. (NY: John Wiley & Sons, Inc.) 3rd edition  
25 (1999), the teachings of which are incorporated herein by reference in their entirety). Protective groups are used to selectively protect reactive groups such as hydroxyl, amino, carboxyl, carbonyl, sulfhydryl, and phosphate groups.

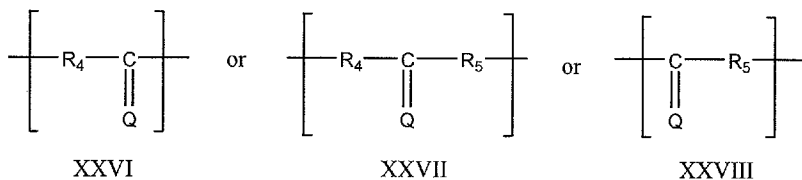
$R_1$  can be a covalent bond, or when  $R_1$  is not a covalent bond,  $R_1$  is also referred to herein as a linker or linker arm.  $R_2$  is also referred to herein as a linker or a linker arm.  
30 A linker can be selected from any suitable alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted, non-substituted, linear or branched group, or a combination of same. Generally, the linker is a hydrophobic linker. For example, the linker can be a 3, 4, 5, 6, 7, 8, 9, 10, or longer carbon chain. Furthermore, a linker used in the invention can be highly hydrophobic or moderately hydrophobic, as will be  
35 understood by one of skill in the art. Alternatively, a hydrophilic linker can be used.

5 In one embodiment, R<sub>1</sub> comprises:



wherein Q = O or NH<sub>2</sub><sup>+</sup>.

In another embodiment, R<sub>1</sub> comprises:

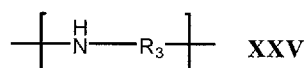


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wherein Q = O or NH<sub>2</sub><sup>+</sup>; R<sub>4</sub> is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof; and R<sub>5</sub> is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof.

15

20 In one embodiment, R<sub>2</sub> comprises the general formula:

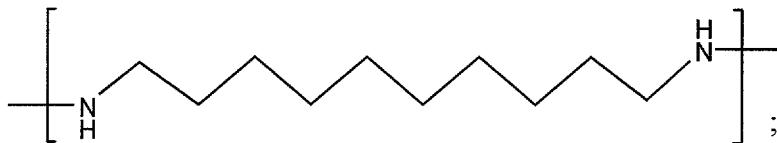
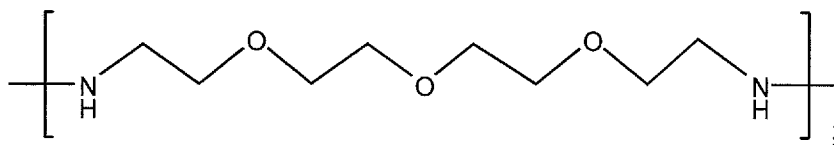
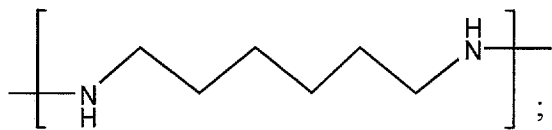
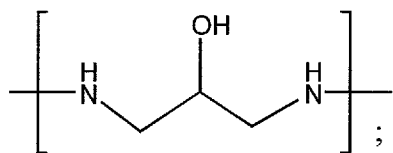


wherein R<sub>3</sub> is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

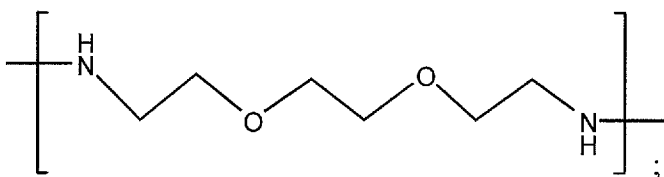
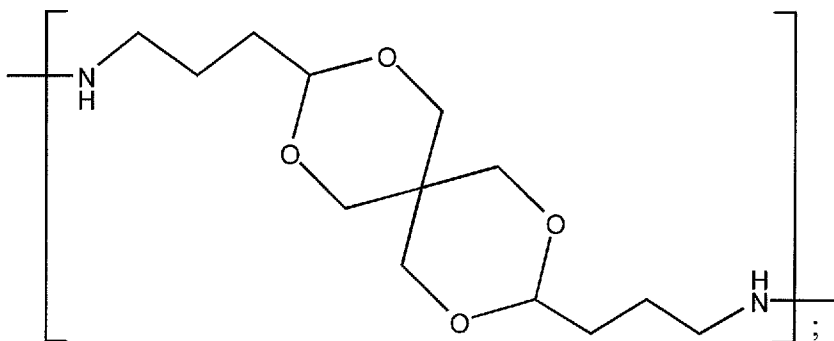
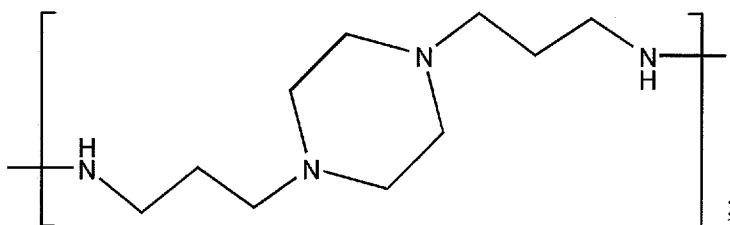
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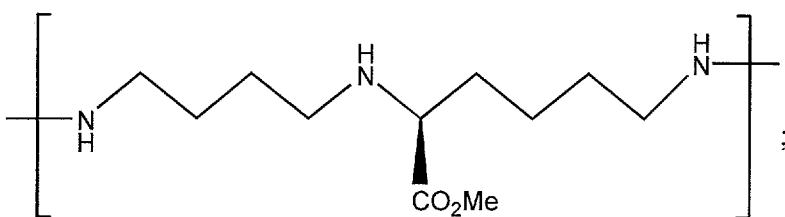
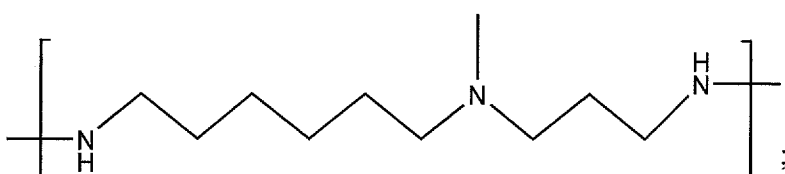
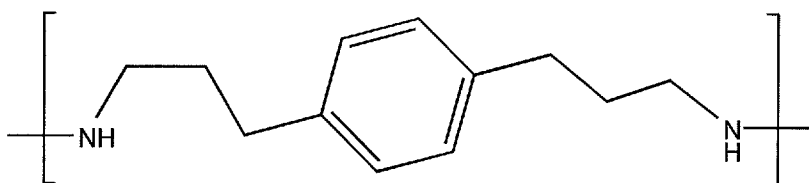
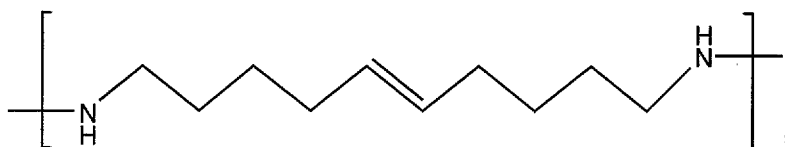
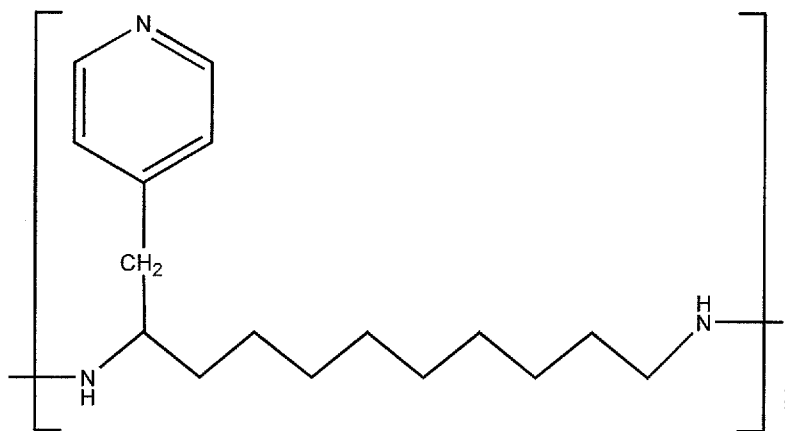
5            Examples of suitable linkers include, without limitation:



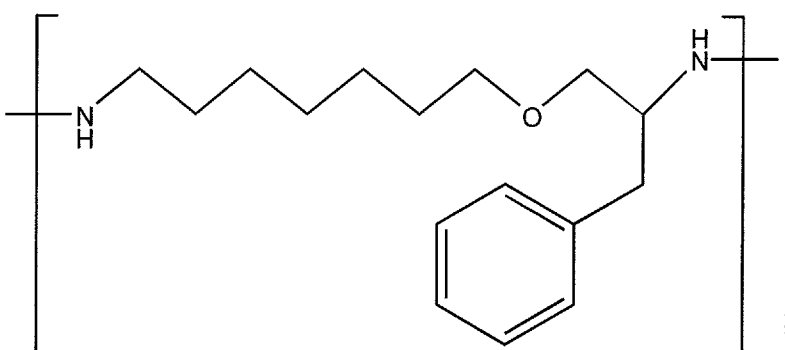
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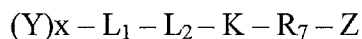
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5           Furthermore, a linker, such as described above, can be attached (for example, via a condensation reaction) to another linker to form a larger and/or a longer linker. For example, a linker can be formed by the tandem synthesis of linkers in a linear configuration. This can be represented, for example, as:

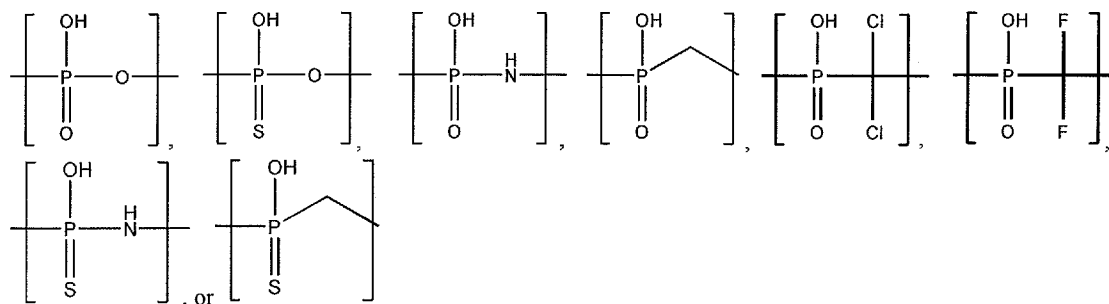


10       where Y is a solid support, a tag or a protective group, x is 0 or 1; L<sub>1</sub> and L<sub>2</sub> are linkers, such as provided in the above examples, and they can be the same or different linkers; K is a heteroatom; R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate and n is at least one or R<sub>7</sub> is a phosphate group mimic, and Z is a nucleoside or nucleoside derivative. As shown in the above examples, two linkers can be synthesized in tandem, however, it will be  
15       understood that two, three, or more linkers, can be synthesized in tandem. Alternatively, a branched configuration of linkers can be synthesized. Again, the linkers can be the same or different. Different linkers can be chosen according to their different hydrophobic or hydrophilic properties, as will be understood by one of skill in the art.

          Additionally or alternatively, more than one alkyl-linked nucleotide can be bound  
20       to Y, for example when Y is a solid support, by more than one type of linker. Again, different linkers can be chosen according to their different hydrophobic or hydrophilic properties, as will be apparent to one of skill in the art. Thus, for example, when Y is a solid support, more than one nucleotide or nucleotide derivative (which may be the same nucleotide or different nucleotides, for example, only ATP, or a mixture of AMP and  
25       ADP, etc.) can be bound to the solid support by linkers that have similar or different hydrophobic properties (or hydrophilic properties). Examples of suitable synthesis methods for these affinity media are provided in the exemplification.

          In some embodiments of the invention, R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate group and n ≥ 1. Examples of suitable phosphate and thiophosphate  
30       groups that may be used include, without limitation:

5

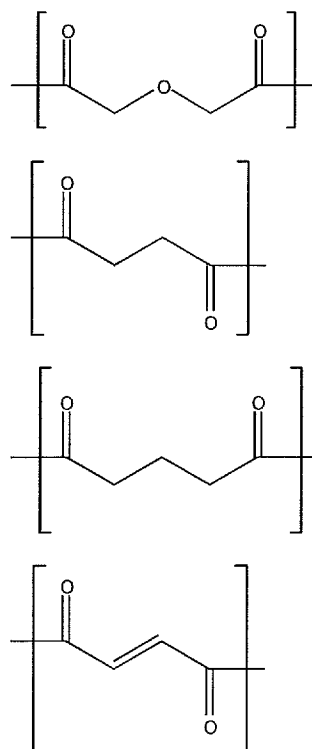


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As will be understood by one of skill in the art, phosphate and thiophosphate groups can also be present in an ionized variant or salt form. In some embodiments of the invention  $n$  is  $\geq 1$ ,  $\geq 2$ ,  $\geq 3$ , or  $\geq 4$ . For example,  $n$  can be 1, 2, 3, or 4. When  $n > 1$ , any combination of phosphate or thiophosphate groups may be used.

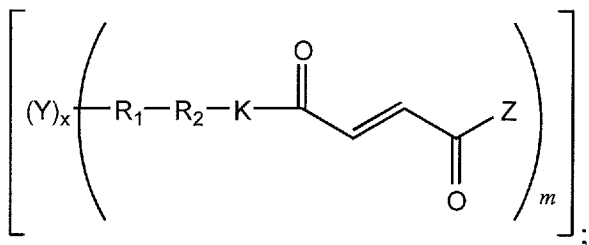
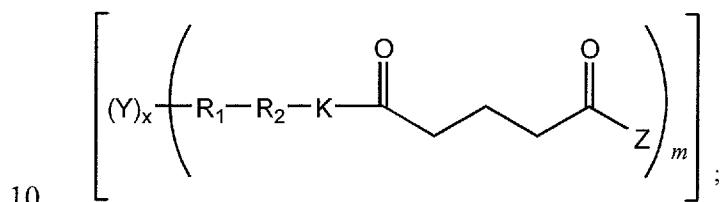
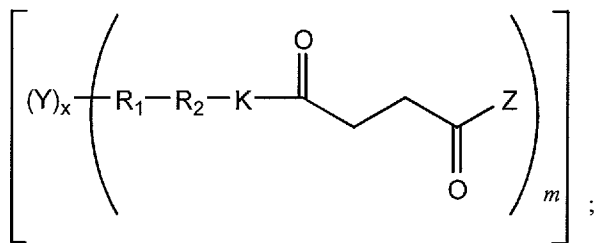
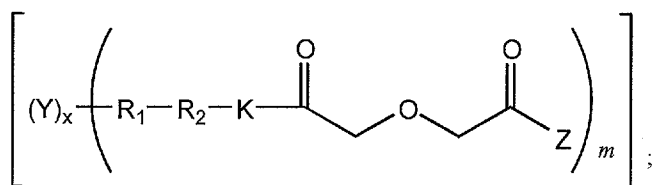
In other embodiments of the invention,  $R_7$  is a phosphate group mimic. For example, in some embodiments,  $R_7$  is a carboxylic acid that contains 4-8 carbons in the main chain and optionally contains a heteroatom. Examples of suitable carboxylic acids include, without limitation:

15

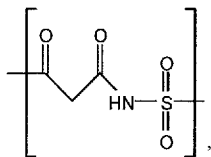
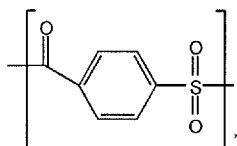


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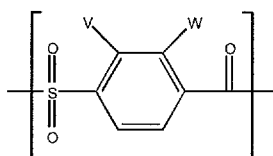
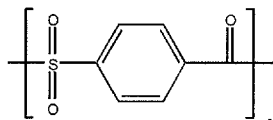
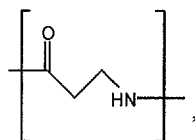
- 5 Accordingly, in some embodiments the alkyl linked nucleotide compositions have a general formula selected from:



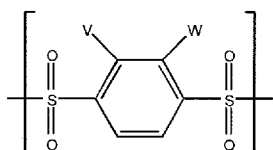
- 15 Other phosphate group mimics that may be used according to the invention include, without limitation, the following:



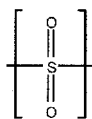
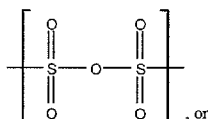
5



where V and W can be H or a heteroatom such as NH<sub>2</sub> or OH,



where V and W can be H or a heteroatom such as NH<sub>2</sub> or OH,



10

A nucleoside and a nucleotide, as referred to herein, can be naturally-occurring or non-naturally-occurring. Furthermore, the nucleoside or nucleotide can be biologically or synthetically-derived using techniques that are standard to one of skill in the art. Suitable nucleosides include, without limitation, adenosine (A), guanosine (G), cytidine (C), thymidine (T) and uridine (U), and derivatives and analogs thereof.

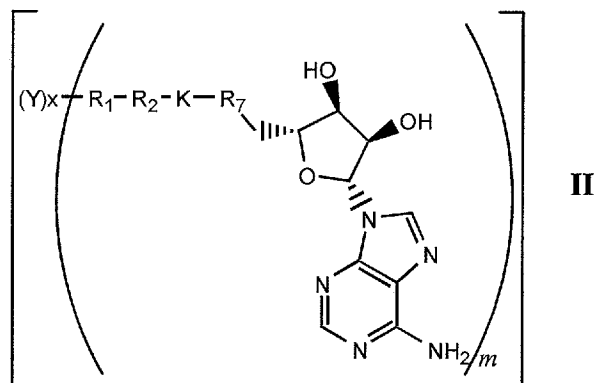
Nucleotides are nucleosides with at least one phosphate group (or thiophosphate group), for example, a monophosphate, diphosphate or triphosphate group. The nucleotide can have phosphate or thiophosphate groups, or a combination of both. The number of phosphate or thiophosphate groups is at least one, and can be one, two, three or more in number. Such nucleotides are often referred to in abbreviation, for example, AMP, ADP, ATP, GMP, GDP, GTP, etc., as is understood by one of skill in the art.

5           In one embodiment, the nucleotide is a monophosphate, diphosphate, or triphosphate of adenosine, guanosine, cytidine, thymidine, or uridine.

          Nucleoside and nucleotide derivatives and analogs are also encompassed by the invention. The isolation or synthesis of nucleoside derivatives and analogs are accomplished using techniques that are standard in the art, see for example, Guranowski  
10 et al. (1981) *Biochemistry* 20:110-15; Yaginuma *et al.* (1981) *J. Antibiot.* 23:359-66; Robins *et al.* (1983) *J. Am. Chem. Soc.* 105:4059-65; Borchardt *et al.* (1984) *J. Biol. Chem.* 259:5353-58; De Clercq *et al.* (1987) *Biochem. Pharmacol.* 36:2567-75; Seela *et al.* (1991) *Helv. Chim. Acta* 74:1048; Franchetti *et al.*, (1994) *J. Med. Chem.* 37: 3534-3541; Van Calenberg *et al.* (1994) *Helv. Chim. Acta.* 77:631-44; Picher *et al.* (1996)  
15 *Biochem. Pharmacol.* 51:1453- 601; Rosse *et al.* (1997) *Helv. Chim. Acta.* 80:653; Cowart *et al.* (1999) *J. Org. Chem.* 64:2240-49. Fischer *et al.*, (1999) *J. Med. Chem.* 42:3636-3646; van Tilburg *et al.* (1999) *J. Med. Chem.* 43:1393- 400; Halbfinger *et al.*, (1999) *J. Med. Chem.* 42:5325-5337; Ingall *et al.* (1999) *J. Med. Chem.* 42:213-20; Gendron *et al.*, (2000) *J. Med. Chem.* 43:2239-2247; Loog *et al.* (2000) *FEBS Letters*  
20 480:244; Bressi *et al.* (2001) *J. Med. Chem.* 44:2080-93; Herforth *et al.* (2002) *J. Comb. Chem.* 4:302-14; Hernandez *et al.* (2002) *J. Med. Chem.* 45:4254-63; Parang *et al.* (2002) *Pharmacology and Therapeutics* 93:145; Xu *et al.* (2002) *J. Med. Chem.* 45:5694-709; Hocek and Dvorakova (2003) *J. Org. Chem.* 68:5773-6; Koroniak *et al.* (2003) *Pharmacology & Therapeutics* 93:145; and Kourafalos *et al.* (2003) *J. Org. Chem.*  
25 68:6466-69; the teachings of all of which are incorporated herein by reference in their entirety.

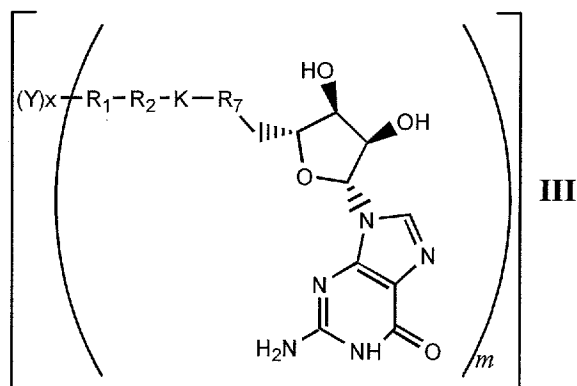
          In one embodiment, the invention includes an alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support comprising an alkyl-linked adenosine, said alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support comprising the general structure:

5



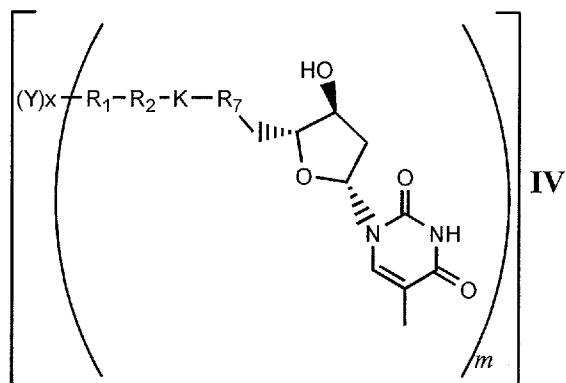
or an ionized variant or a salt thereof.

In another embodiment is an alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support comprising an alkyl-linked guanosine, said alkyl-linked  
 10 nucleotide ~~composition~~ non-homogeneous solid support comprising the general structure:



or an ionized variant or a salt thereof.

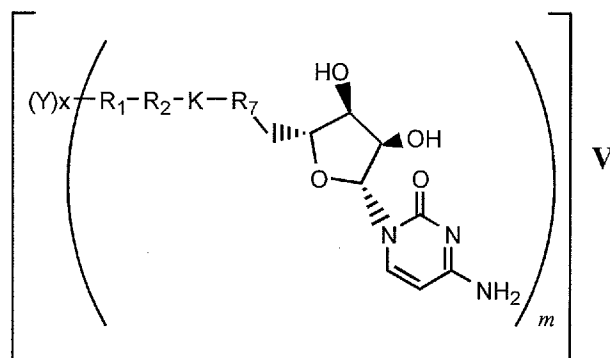
In another embodiment is an alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support comprising an alkyl-linked thymidine, said alkyl-linked  
 15 nucleotide ~~composition~~ non-homogeneous solid support comprising the general structure:





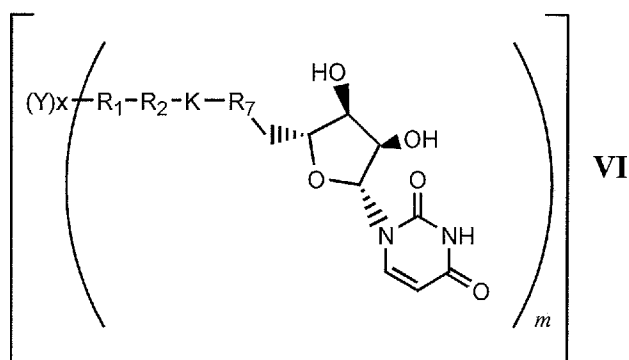
5 or an ionized variant or a salt thereof.

In a further embodiment is an alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support comprising an alkyl-linked cytidine, said alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support comprising the general structure:



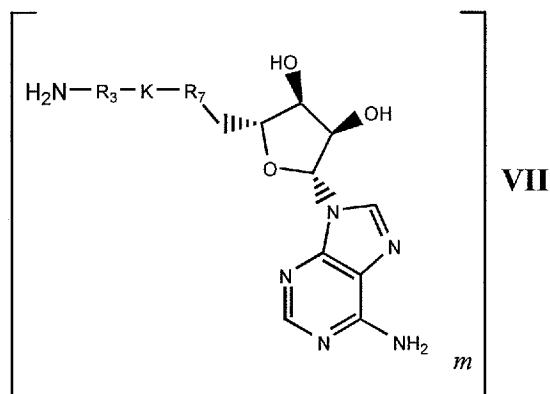
10 or an ionized variant or a salt thereof.

In yet another embodiment is an alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support comprising an alkyl-linked uridine, said alkyl-linked nucleotide ~~composition~~ non-homogeneous solid support comprising the general structure:



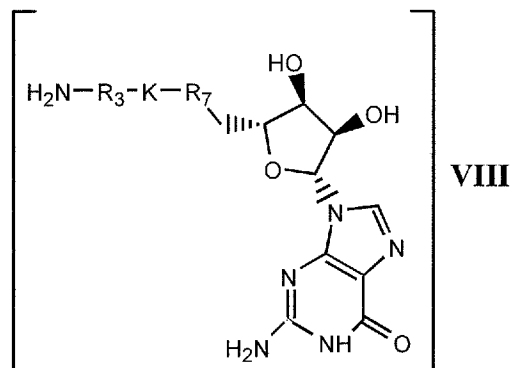
15 or an ionized variant or a salt thereof.

In another embodiment, the invention includes an alkyl-linked adenosine comprising the general structure:



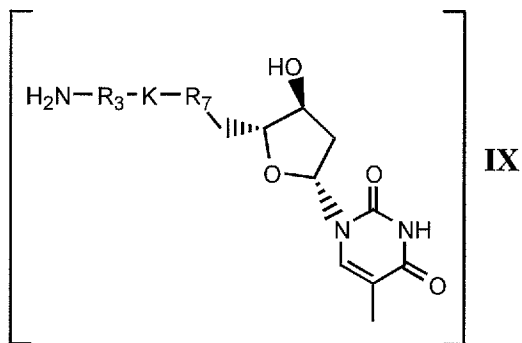
5 or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination  
10 thereof.

In further embodiment, the invention includes an alkyl-linked guanosine, comprising the general structure:



15 or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

20 In another embodiment, the invention includes an alkyl-linked thymidine comprising the general structure:

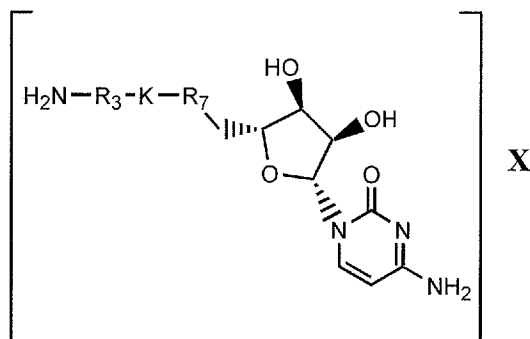


5

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In yet another embodiment, the invention includes an alkyl-linked cytidine comprising the general structure:

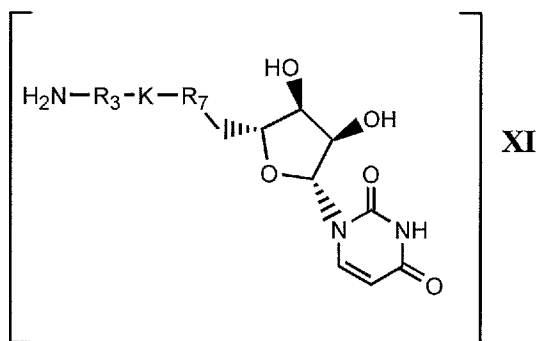


15

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

In a further embodiment, the invention includes an alkyl-linked uridine comprising the general structure:

20

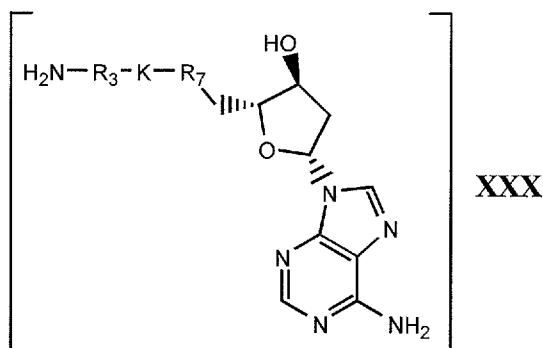


5

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In another embodiment, the invention includes an alkyl-linked 2'-deoxy-adenosine comprising the general structure:

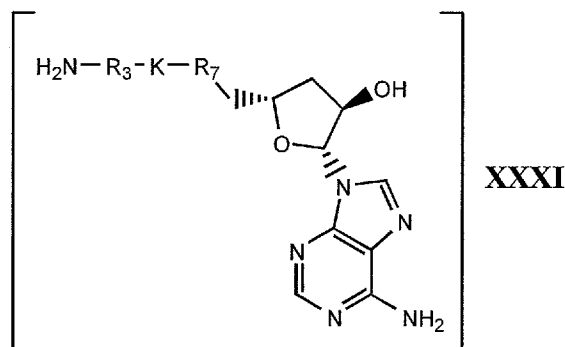


15

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

20

In another embodiment, the invention includes an alkyl-linked 3'-deoxy-adenosine comprising the general structure:

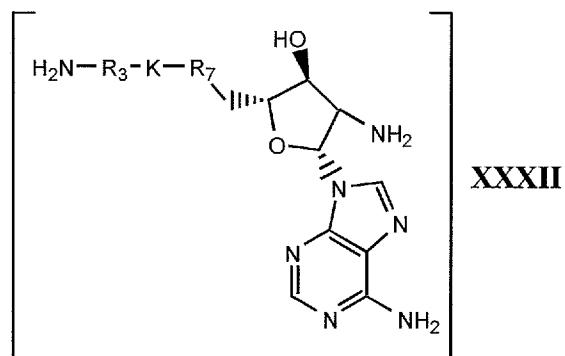


5

or an ionized variant or a salt thereof, wherein the linker (R<sub>3</sub>) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In another embodiment, the invention includes an alkyl-linked 2'-deoxy-2'-amino-adenosine comprising the general structure:

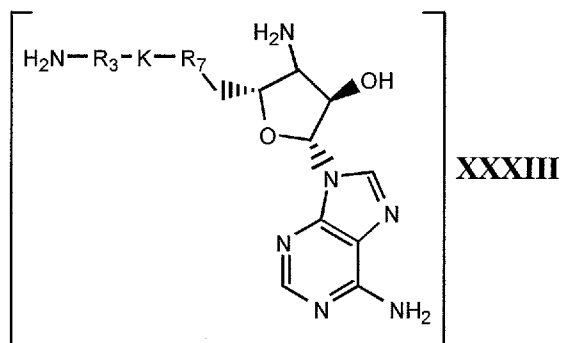


15

or an ionized variant or a salt thereof, wherein the linker (R<sub>3</sub>) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

20

In another embodiment, the invention includes an alkyl-linked 3'-deoxy-3'-amino-adenosine comprising the general structure:

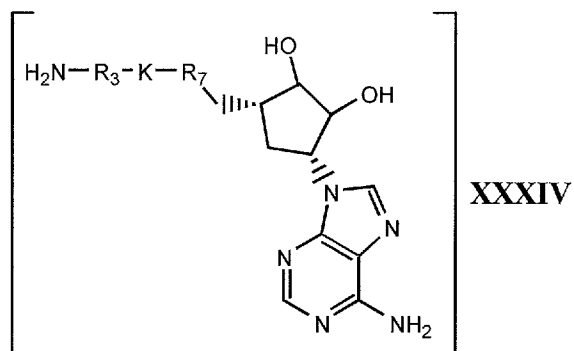


5

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In another embodiment, the invention includes an alkyl-linked adenosine derivative, Aristeromycin comprising the general structure:

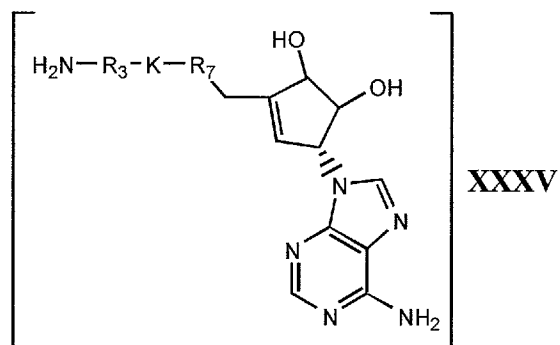


15

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

In another embodiment, the invention includes an alkyl-linked ATP derivative, Neplanocin A, comprising the general structure:

20

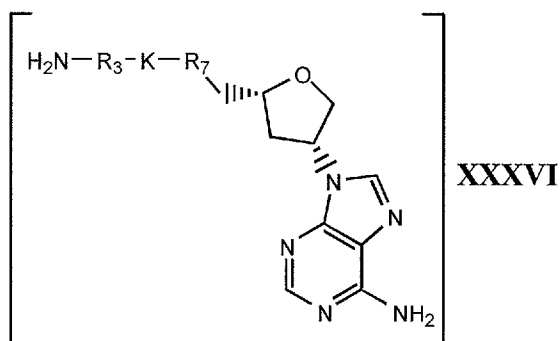


5

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In another embodiment, the invention includes an alkyl-linked 2',3'-dideoxy-3'-oxoadenosine, comprising the general structure:



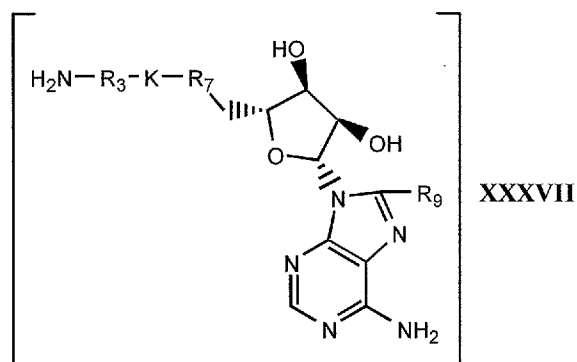
or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

15

In other embodiments, the invention includes an alkyl-linked 2-, 6-, or 8-substituted adenosine derivative. These substituted adenosine derivatives can be made by the condensation of the corresponding bromides (for the 2 and 8 position) or chlorides (for the 6 position) and the appropriate amine (including, for example, allylamine, benzylamine, t-butylamine, 2-methoxyethylamine, and diethylamine. See, for example Halbfinger *et al.* (1999) *J. Med. Chem.* 42:5325-37 and van Tilburg *et al.* (1999) *J. Med.*

20

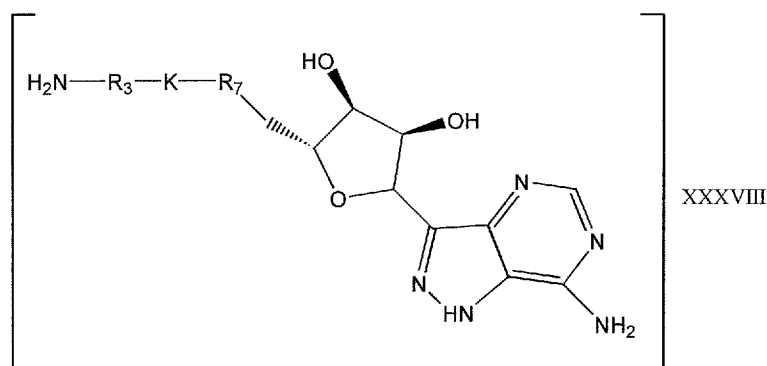
- 5 *Chem.* 42:1393-400; each of which is herein incorporated by reference in its entirety. An example of the general structure of alkyl -linked 8-substituted adenosine is shown below:



- or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof, and  $R_9$  is an amine.
- 10

In an additional embodiment, the invention includes an alkyl-linked formycin A.

- 15 An example of the general structure of alkyl -linked formycin A is shown below:

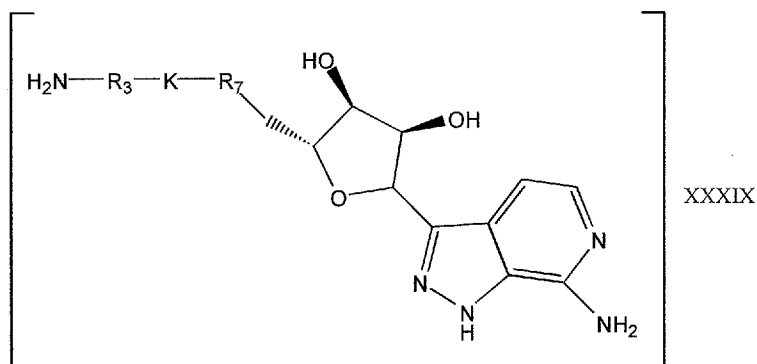


- or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.
- 20

In an additional embodiment, the invention includes an alkyl-linked 4-deazaformycin. The synthesis of 4-deazaformycin A is described in Kourafalos *et al.*

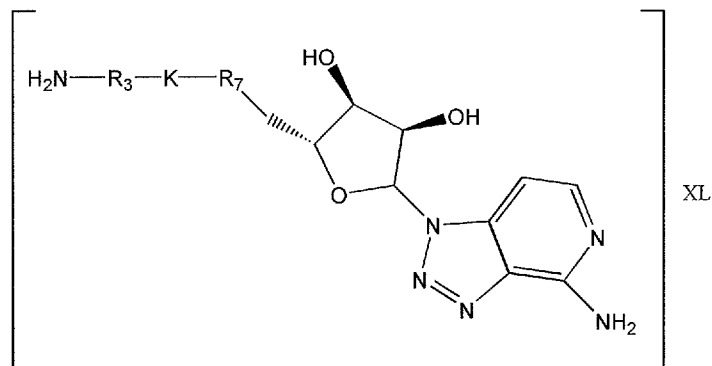


5 (2003) *J. Org. Chem.* 68:6466-69, herein incorporated by reference. An example of the general structure of alkyl -linked 4-deazaformycin A is shown below:



or an ionized variant or a salt thereof, wherein the linker (R<sub>3</sub>) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted  
10 or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

In some embodiments, the invention includes an aza or deaza adenosine derivative. The synthesis of 8-aza, 8-aza-1-deaza, 8-aza-3-deaza, 1-deaza, 3-deaza, and  
15 1,7-deaza adenine derivatives is described in Franchetti *et al.* (1994) *J. Med. Chem.* 37, 3534 and Seela *et al.* (1991) *Helv. Chim. Acta* 74:1048. These derivatives can be reacted with sugar halides or  $\beta$ -D-ribofuranose-1-acetate-2,3,5-tribenzoate (*see*, Kraybill *et al.* (2002) *J. Am. Chem. Soc.* 124:12118 and Saneyoshi *et al.* (1979) *Chem. Pharm. Bull.* 27:2518) and SnCl<sub>4</sub> to form additional adenosine derivatives. An example of the general  
20 structure of one such derivative is shown below:

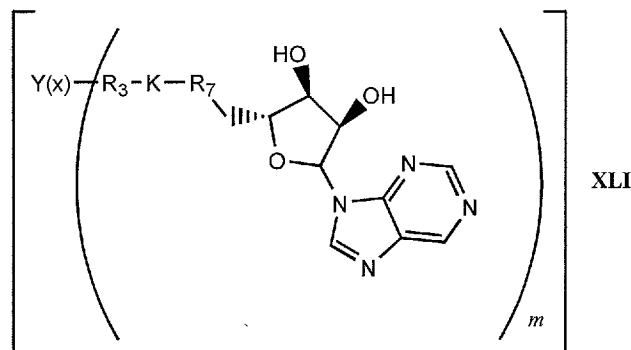


or an ionized variant or a salt thereof, wherein the linker (R<sub>3</sub>) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted

5 or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

In a further embodiment, the invention includes an alkyl-linked purine riboside.

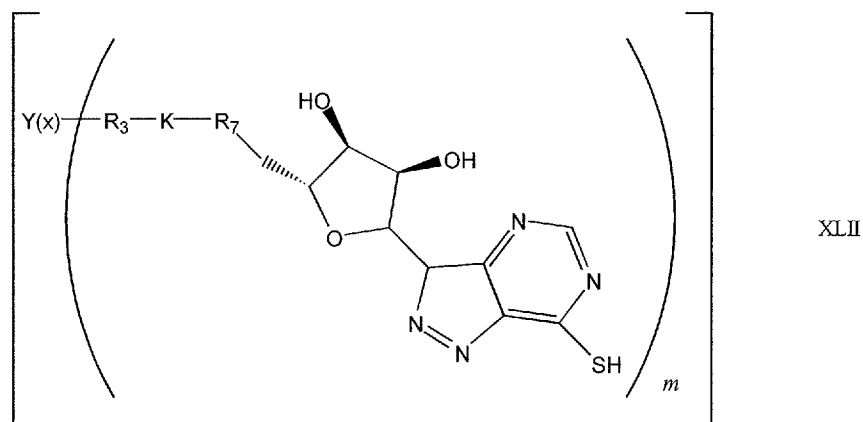
10 An example of the general structure of alkyl-linked purine riboside is shown below:



or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

15

The invention also provides an alkyl-linked 6-mercaptapurine riboside. An example of the general structure of alkyl-linked 6-mercaptapurine riboside is shown below:

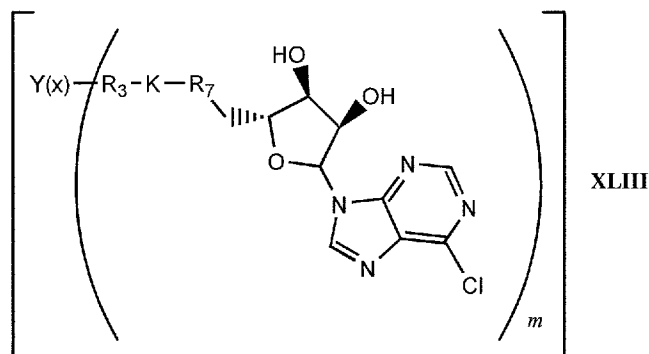


20

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted

5 or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

The invention also provides an alkyl-linked 6-chloropurine riboside. An example of the general structure of alkyl-linked 6-chloropurine riboside is shown below:



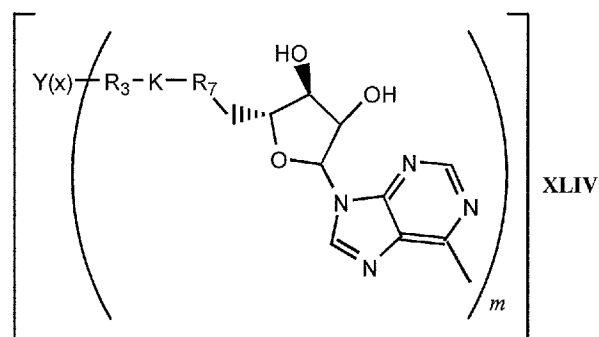
10

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

15

The invention also provides an alkyl-linked 6-methyl purine riboside. The 6-methyl purine riboside may be synthesized by reacting 6-chloropurine riboside with the Grignard reagent methyl magnesium chloride to yield 6-methyl purine riboside (Hocék and Dvoraková (2003) *J. Org. Chem.* 68:5773-6, herein incorporated by reference). An example of the general structure of alkyl-linked 6-methyl purine riboside is shown below:

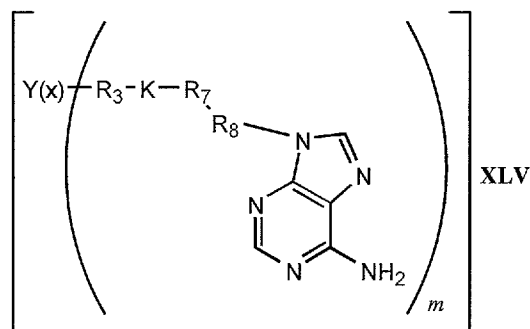
20



or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted

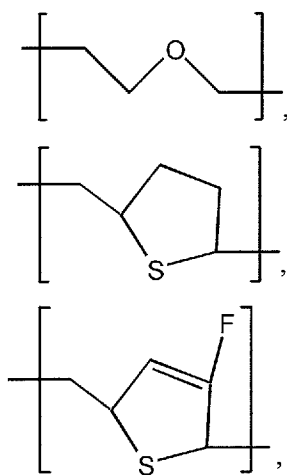
5 or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

In other embodiments, the invention includes an alkyl-linked adenosine derivative in which the ribose group has been replaced with a ribose mimic. One example of the  
10 general structure of such an alkyl-linked derivative is

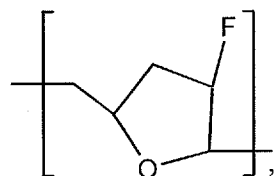
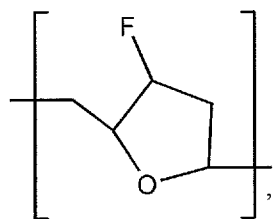
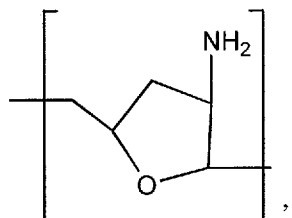
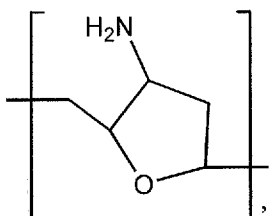
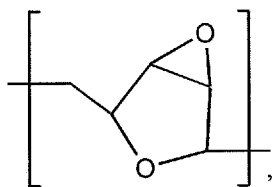
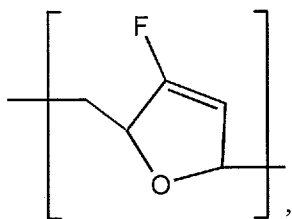
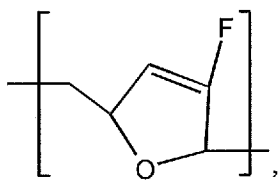


or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted  
15 heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof and  $R_8$  is a ribose mimic. In some embodiments, the ribose mimic is an alkyl,  $C_4$ - $C_7$  cycloalkyl, heteroalkyl, aryl, or heteroaryl group. See, for example Hernandez *et al.* (2002) *J. Med. Chem.* 45:4254-63, herein incorporated by reference in its entirety.

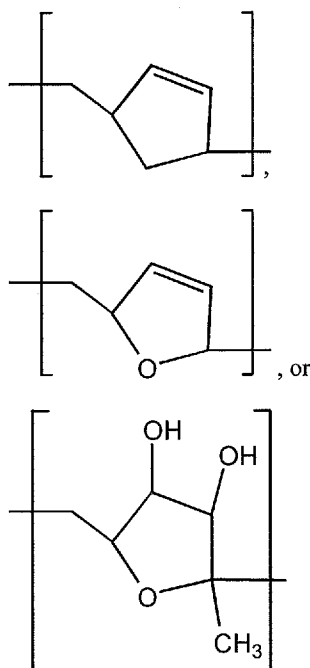
Examples of other suitable ribose mimics include, without limitation:



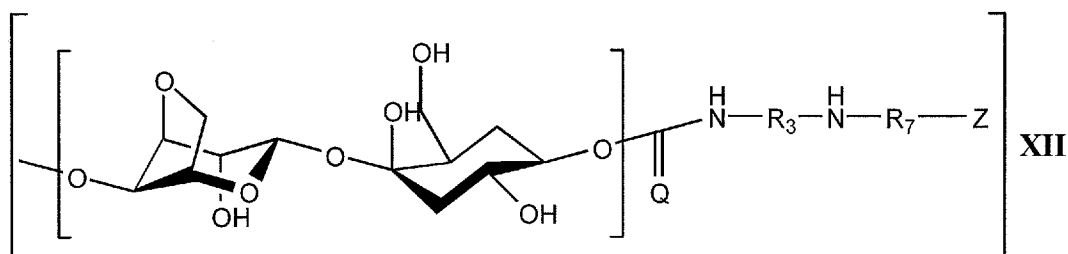
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5

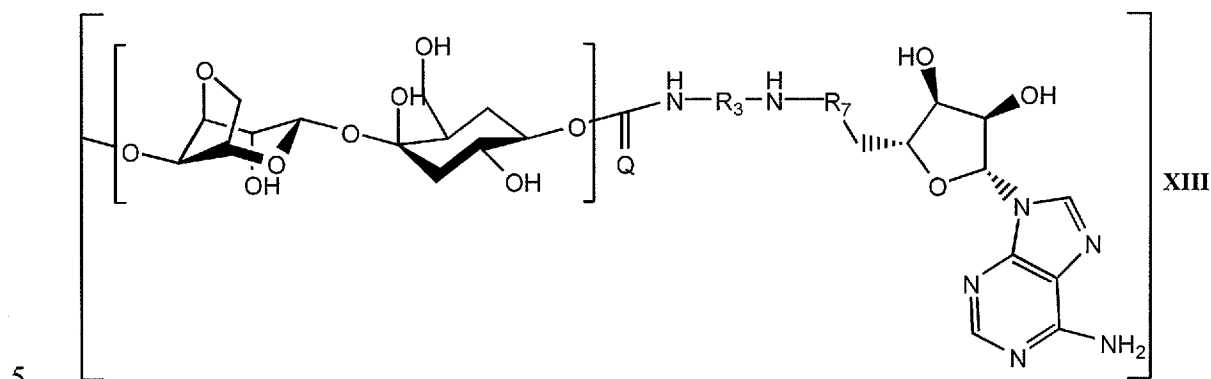


10 In another embodiment is an alkyl-linked nucleotide covalently bound to agarose comprising the general structure:



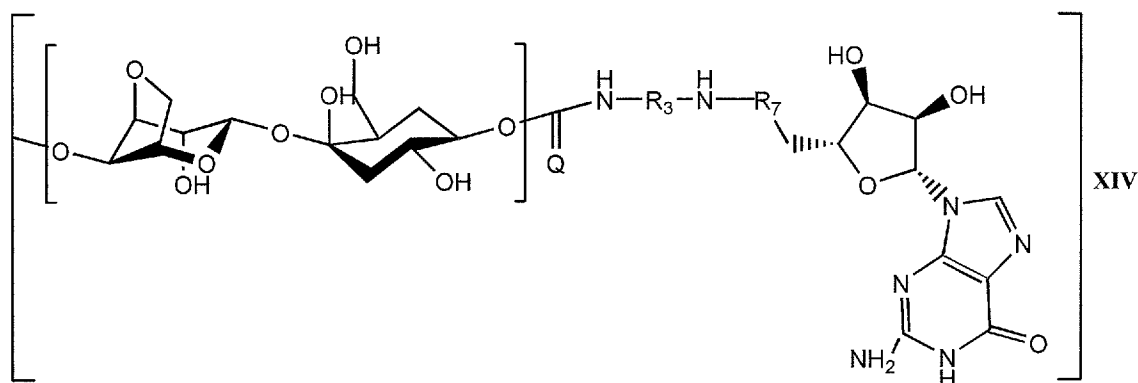
or an ionized variant or a salt thereof, wherein Q = NH<sub>2</sub><sup>+</sup> or O.

15 In one embodiment, the invention includes an alkyl-linked adenosine covalently bound to agarose comprising the general structure:



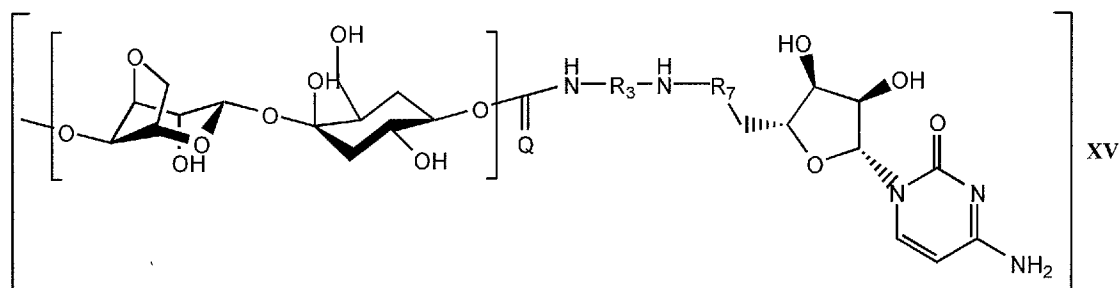
or an ionized variant or a salt thereof, wherein  $Q = \text{NH}_2^+$  or  $\text{O}$ .

In one embodiment, the invention includes an alkyl-linked guanosine covalently bound to agarose comprising the general structure:



10 or an ionized variant or a salt thereof, wherein  $Q = \text{NH}_2^+$  or  $\text{O}$ .

In one embodiment, the invention includes an alkyl-linked cytidine covalently bound to agarose comprising the general structure:



or an ionized variant or a salt thereof, wherein  $Q = \text{NH}_2^+$  or  $\text{O}$ .

15 In one embodiment, the invention includes an alkyl-linked thymidine covalently bound to agarose comprising the general structure:



In one embodiment, the invention includes an alkyl-linked uridine covalently bound to agarose comprising the general structure:

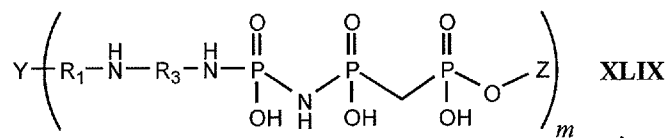


Also included in the invention are  $\gamma$ -alkyl-linked nucleotide triphosphates comprising the general formula:

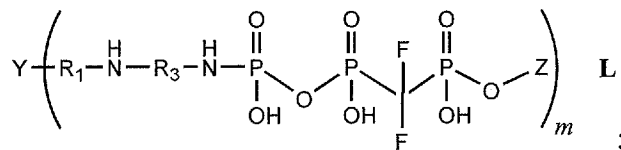




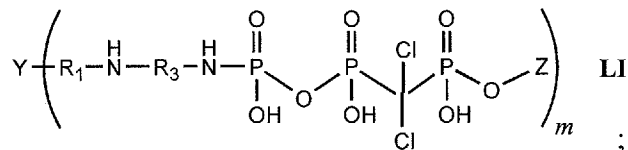
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;



;

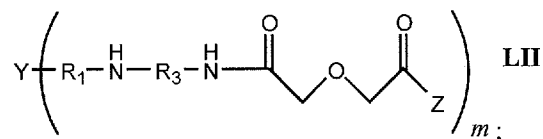


;

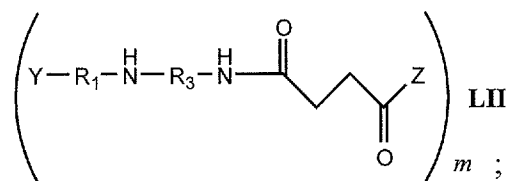
or an ionized variant or a salt thereof.

10

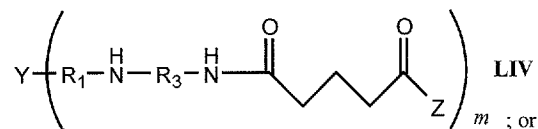
Also included in the invention are  $\gamma$ -alkyl-linked nucleotide analogs comprising the general formula:



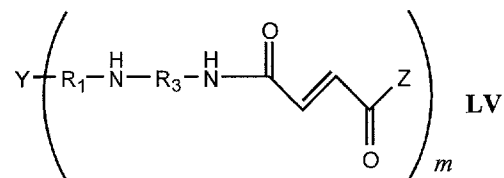
;



;



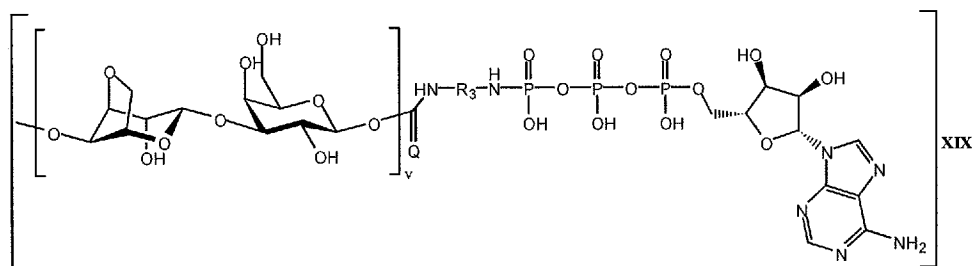
; or



15

In one embodiment, the invention includes a  $\gamma$ -phosphate-linked adenosine triphosphate bound to an agarose solid support comprising the general formula:

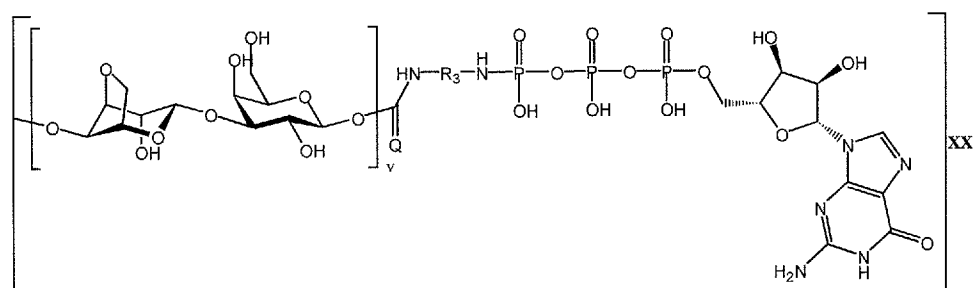
5



or an ionized variant or a salt thereof.

In one embodiment, the invention includes a  $\gamma$ -phosphate-linked guanosine triphosphate bound to an agarose solid support comprising the general formula:

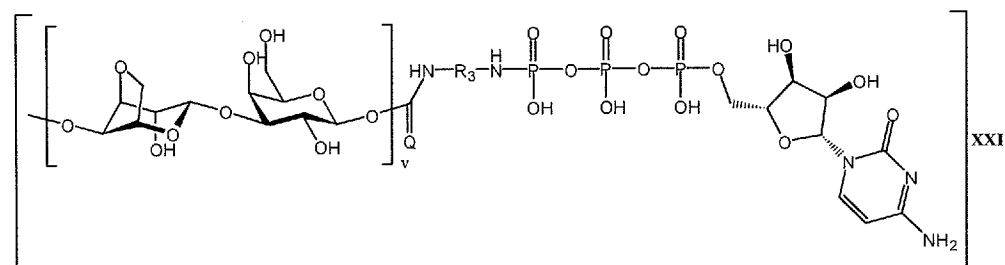
10



or an ionized variant or a salt thereof.

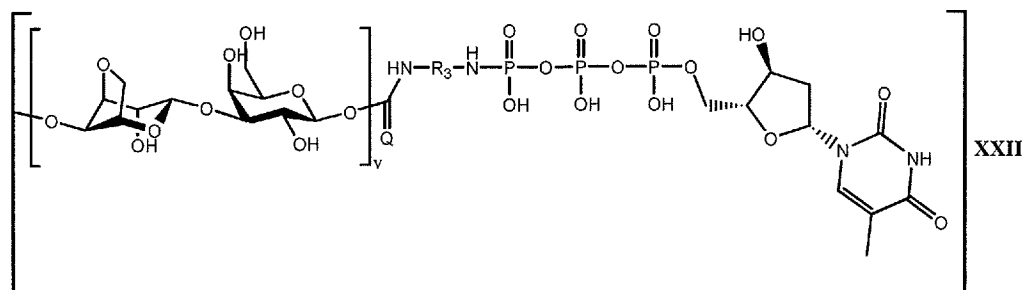
In another embodiment, the invention includes a  $\gamma$ -phosphate-linked cytidine triphosphate bound to an agarose solid support comprising the general formula:

15



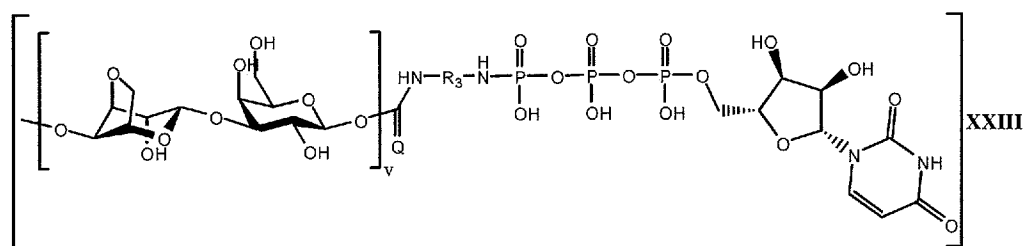
or an ionized variant or a salt thereof.

In a further embodiment, the invention includes a  $\gamma$ -phosphate-linked thymidine triphosphate bound to an agarose solid support comprising the general formula:



or an ionized variant or a salt thereof.

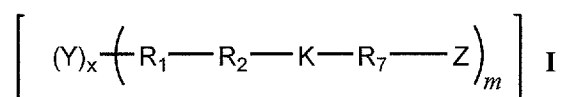
In another embodiment, the invention includes a  $\gamma$ -phosphate-linked uridine triphosphate bound to an agarose solid support comprising the general formula:



or an ionized variant or a salt thereof.

### Synthesis of Alkyl-linked Nucleotide Affinity Media

Also included in the invention is a method to synthesize an alkyl-linked nucleotide affinity medium comprising a general formula:



comprising the general steps of (a) coupling at least one linker to a solid support or tag in a suitable coupling buffer, wherein the linker is  $R_2$ , or a combination of  $R_1$  and  $R_2$ ; (b) end-capping reactive sites remaining on the solid support or tag after the coupling step; and (c) reacting a terminal phosphate or thiophosphate group of a nucleotide with the linker coupled to the solid support or tag, wherein Y is a solid support or a tag;  $x = 1$ ;  $R_1$  is a covalent bond between Y and  $R_2$ , or  $R_1$  is an acyl group, a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof;  $R_2$  is a substituted or a non-substituted alkyl

5 group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof; K is a heteroatom;  $R_7$  is  $(P)_n$  where P is a phosphate or thiophosphate and n is at least one or  $R_7$  is a phosphate group mimic, Z is a nucleoside or nucleoside derivative; and m is at least  
10 one. As described elsewhere herein, a solid support can be any suitable support, such as a resin, or a particulate material, such as a bead, or a particle. Alternatively, a solid support can be a continuous solid surface, such as a plate, chip, well, channel, column or a tube. The material of a solid support will be of any suitable substance, compound or polymer, as will be appreciated by one of skill in the art. Examples include, without  
15 limitation, acrylamide, agarose, methacrylate polymers, methacrylate co-polymers, thermoresponsive polymers, cellulose, nylon, silica, glass, ceramic, a magnetized particle or surface, nitrocellulose, polystyrene and derivatives thereof.

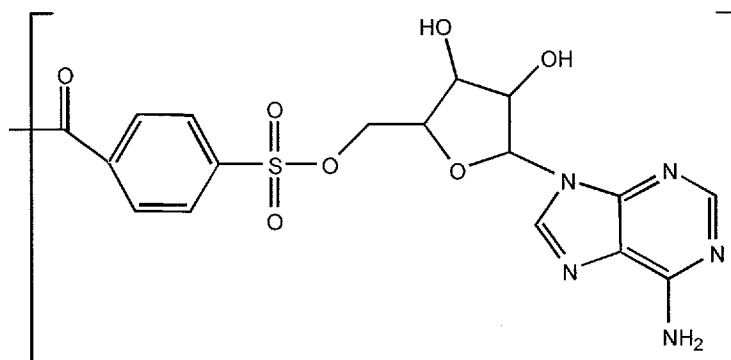
Furthermore, a tag, as used herein, is an agent that provides for the specific detection or capture of the alkyl-linked nucleotide. For example, and without limitation,  
20 a suitable tag is biotin, avidin, streptavidin, a hapten, a fluorophore or a chromophore. Detection or capture of the alkyl-linked nucleotide employs techniques that are standard in the art. For example, a biotin-tagged alkyl-linked nucleotide can be captured using avidin, streptavidin or related avidin derivatives. The biotin-avidin interaction is highly specific. An avidin-conjugated agent used to detect or capture a biotin-tagged alkyl-  
25 linked nucleotide can be soluble (for example, an antibody), or a particulate material (for example, beaded agarose or a magnetized particle), or a continuous surface (for example, a plate or well coated with avidin). Detection and capture of a hapten-tagged alkyl-linked nucleotide can be achieved, for example, using hapten-specific antibodies. Visual  
30 detection methods for fluorophore or chromophore-tagged alkyl-linked nucleotides are readily understood by one of skill in the art.

A nucleoside and a nucleotide, as referred to herein, can be naturally-occurring or non-naturally-occurring. Furthermore, the nucleoside or nucleotide can be biologically or synthetically-derived using techniques that are standard to one of skill in the art. Suitable  
35 nucleosides include, without limitation, adenosine (A), guanosine (G), cytidine (C), thymidine (T) and uridine (U), and derivatives thereof.

5 In one embodiment, the nucleotide is a monophosphate, diphosphate, triphosphate, or tetraphosphate of adenosine, guanosine, cytidine, thymidine, or uridine or an analog thereof. In certain embodiments, the phosphate moiety of the nucleotide is modified. See, for example Picher *et al.* (1996) *Biochem. Pharmacol.* 51:1453-60; Ingall  
10 *et al.* (1999) *J. Med. Chem.* 42:213-20; Gendron *et al.* (2000) *J. Med. Chem.* 43:2239-47; and Xu *et al.* (2002) *J. Med. Chem.* 45:5694-709 each of which is herein incorporated in its entirety by reference.

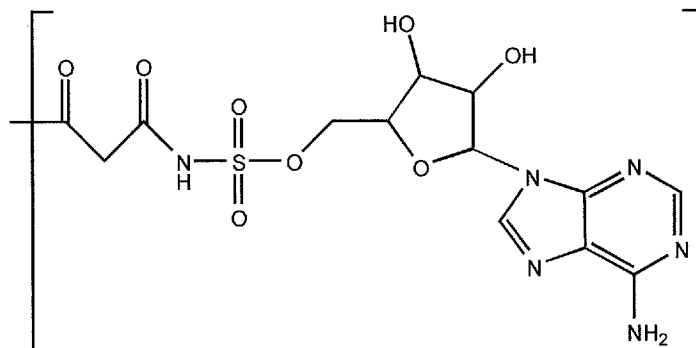
In other embodiments, the phosphate moiety of the nucleotide is replaced by a phosphate group mimic. For example, in some embodiments, the alkyl-linked nucleotide  
15 compositions non-homogeneous solid supports comprise a carboxylic acid that contains 4-8 carbons in the main chain and optionally contains a heteroatom. To make these nucleotide derivatives, 2',3'-isopropylidene adenosine is reacted with a cyclic anhydride, and then the acetonide is deprotected. Examples of cyclic anhydrides that may be reacted with 2',3'-isopropylidene include diglycolic anhydride, succinic anhydride, glutaric anhydride, and maleic anhydride, although any suitable electrophile may be used.  
20 The functionality of the 5'-hydroxyl of adenosine is may also be converted into a primary amine by a Mitsunobu reaction with phthalimide followed by hydrazinolysis. See, for example Bressi *et al.* (2001) *J. Med. Chem.* 44:2080-93 and Herforth *et al.* (2002) *J. Comb. Chem.* 4:302-14; each of which are herein incorporated by reference in their entirety.

25 Other phosphate group mimics may be used according to invention. For example, an ATP analog can be made by reacting 2',3'-O-isopropylidene-adenosine with 4-chlorosulfonylbenzoic acid to give an ATP analog having the formula:



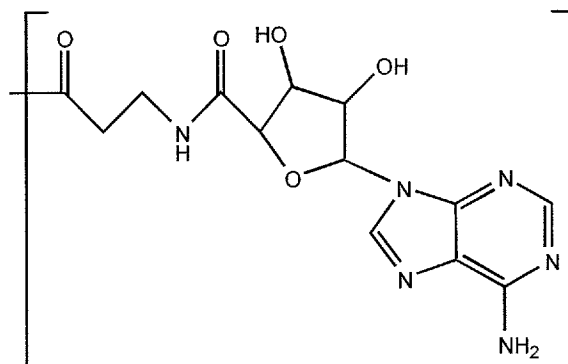
- 5 The benzene ring can be substituted with hydroxyl or amine groups. *See*, for example, Rosse *et al.* (1997) *Helv. Chim. Acta.* 80:653, herein incorporated by reference.

In another example, 2',3'-O-isopropylidene-adenosine is reacted with sulfamoyl chloride, the sulfonamide is conjugated with a carboxylic acid (for example, benzyl malonate or *t*-butyl malonate) and then deprotected in one or two steps to give an ATP  
10 analog having the structure:



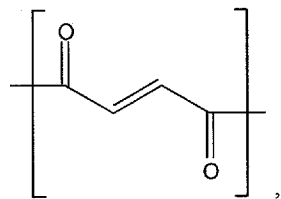
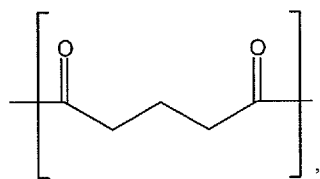
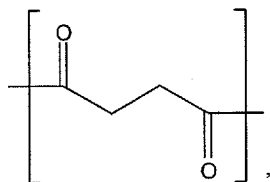
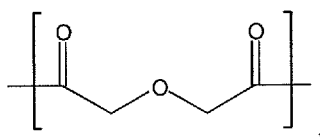
After conjugation with *t*-butyl malonate only one acidic deprotection step is performed; After conjugation with benzyl malonate a catalytic hydrogenation of the benzyl group  
15 and an acidic cleavage of the acetonide group are performed. In addition to mono-protected malonates, mono-protected succinates or glutarates can be used. In addition, the sulfonamide can be reacted with other electrophiles such as bromoacetic acid to obtain phosphate group mimics. Amino acids can also be used to link the sulfonamide with a  
20 linker. *See*, Koroniak *et al.* (2003) *Pharmacology & Therapeutics* 93:145, herein incorporated by reference.

In a further example, an ATP analog is made by conjugating adenosine-5'-carboxylic acid with  $\beta$ -alanine to form an ATP analog having the structure:

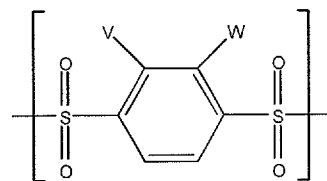
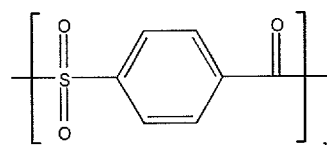


- 5 Other aminocarboxylic acids can be used. In addition, adenosine-5'-carboxylic acid can be conjugated with amino acids or peptides. See, Loog *et al.* (2000) *FEBS Letters* 480:244; and Parang *et al.* (2002) *Pharmacology and Therapeutics* 93:145, both of which are herein incorporated by reference in their entirety.

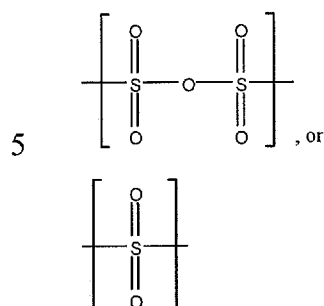
- 10 Other non-limiting examples of phosphate group mimics that may be used in the alkyl-linked nucleotide compositions non-homogeneous solid supports of the present invention include:



15



where V and W can be H or a heteroatom such as NH<sub>2</sub> or OH,



The ribose moiety of the alkyl-linked nucleotide may be modified or replaced. For example, oxidation and acetonide protection of 4-penten-1-ol yields an intermediate  
 10 that can be reacted with the adenine under classic Mitsunobu conditions. Mitsunobu reactions between alcohols and the adenine N9 are well established. *See*, for example, Chang *et al.* (1999) *Chemistry & Biology* 6:361-75. Deprotection of the acetonide then leads to a first ligand ready for phosphorylation and attachment to a resin-linker arm combination. The primary alcohol of the same intermediate can also be oxidized to the  
 15 aldehyde, followed by acetonide deprotection and ring closure. Selective protection of the primary hydroxyl over the secondary hydroxyl, a Mitsunobu reaction, and deprotection of the primary hydroxyl results in a second ligand.

Non-limiting examples of nucleoside analogs that may be used in the compositions of the invention include 2'-deoxy-adenosine, 3'-deoxy-adenosine, 2'-deoxy-  
 20 2'-amino-adenosine, 3'-deoxy-3'-amino adenosine, formycin A, 4-deazaformycin A, aristeromycin, neplanocin A, purine riboside, 6-mercaptopurine riboside, 6-chloropurine riboside, 6-methyl purine riboside, and 2',3'-dideoxy-3'-oxoadenosine. *See*, for example, Guranowski *et al.* (1981) *Biochemistry* 20:110; Yaginuma *et al.* (1981) *J. Antibiot.* 23:359; Robins *et al.* (1983) *J. Am. Chem. Soc.* 105:4059; Borchardt *et al.* (1984) *J. Biol.*  
 25 *Chem.* 259:5353; De Clerq *et al.* (1987) *Biochem. Pharmacol.* 36:2567; Huryn *et al.* (1989) *Tetrahedron Lett.* 30:6259; Franchetti *et al.* (1994) *J. Med. Chem.* 37:3534; Van Calenbergh *et al.* (1994) *Helv. Chim. Acta.* 77:631; Cowart *et al.* (1999) *J. Org. Chem.* 64:2240; Hockek and Dvorakova (2003) *J. Org. Chem.* 68:5773; and Kourafalos *et al.* (2003) *J. Org. Chem.* 68:6466-69; each of which is herein encompassed by reference in  
 30 its entirety.



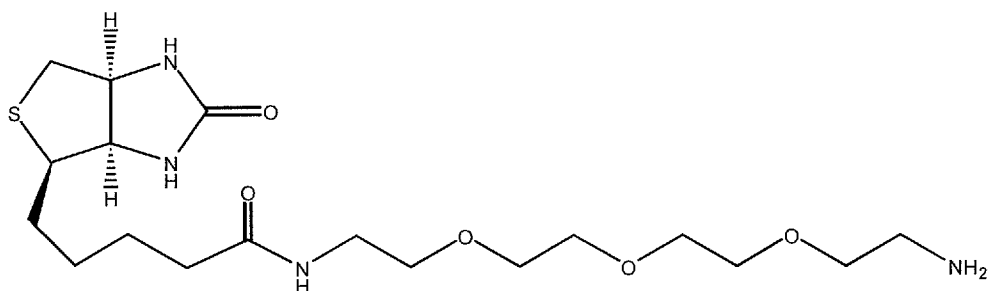
5           In still other embodiments, the nucleoside or nucleotide is a 2-, 6- or 8-substituted adenosine derivative. Such derivatives may be made by the condensation of the corresponding bromides (for the 2 and 8 position) or chlorides (for the 6 position) and the appropriate amine, *e.g.* allylamine, benzylamine, *t*-butylamine, 2-methoxyethylamine, diethylamine. *See*, for example Halbfinger *et al.* (1999) *J. Med. Chem.* 42: 5325, and van Tilburg *et al.* (1999) *J. Med. Chem.* 42:1393.

10           In some embodiments, the compositions comprise a ribose mimic. For example, the ribose of the nucleoside or nucleotide is replaced with an alkyl, C<sub>4</sub>-C<sub>7</sub> cycloalkyl, heteroalkyl, aryl, or heteroaryl group. This may be accomplished by monotitrylation of diols, followed by a Mitsunobu reaction with adenine and deprotection of the titrylated alcohol. *See*, for example, Hernandez *et al.* (2002) *J. Med. Chem.* 45, 4254, herein incorporated by reference. Other ribose mimics and their syntheses are described, for example, in Lee *et al.* (1961) *J. Am. Chem. Soc.* 83:1906; Imazawa (1978) *J. Org. Chem.* 43:3044; Robins *et al.* (1984) *Tetrahedron Letters* 25:367; Herdewijn *et al.* (1987) *J. Med. Chem.* 30:2131-37; Wu *et al.* (1988) *Tetrahedron* 44:6705; Van Aerschot *et al.* (1989) *J. Med. Chem.* 32:1743-49; Secrist *et al.* (1991) *J. Med. Chem.* 34:2361-66; Secrist *et al.* (1992) *J. Med. Chem.* 35:533-38; Holletz *et al.* (1994) *Synthesis* 8:789; Choi *et al.* (1998) *Tetrahedron Letters* 25:367; Meier *et al.* (1999) *Nucleosides Nucleotides* 18:907-12; Meier *et al.* (1999) *J. Med. Chem.* 42:1615-24; and Choo *et al.* (2003) *J. Med. Chem.* 46:389-98.

25           The synthesis of an alkyl-linked nucleotide affinity medium comprising alkyl-linked nucleotides is generally done in three steps: first the linker (or linkers) is attached to the solid support (also referred to herein generally as a resin); second, any remaining active sites on the solid support are end-capped using a suitable reagent such as, for example, ethanolamine, 2-amino-2-(hydroxymethyl)-1,3-propanediol, or glycine; and  
30           third, the linker arm is reacted with the affinity ligand of choice, for example, a nucleotide, such as, adenosine triphosphate (ATP), thus producing a nucleotide affinity medium.

          The attachment of a tag, such as biotin, to a nucleotide via a linker uses techniques that are standard in the art. For example, biotin can be synthesized with a

5 linker arm attached; such compounds are known to one of skill in the art and are commercially available with different linker arms already attached; for example:



To synthesize an alkyl-linked nucleotide attached to a biotin tag, the water-soluble biotin  
linker complex is reacted with the nucleotide which has been reacted with 1-(3-  
10 dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-methyl  
imidazole.

The synthesis of an alkyl-linked nucleotide with a protective group uses  
techniques that are known in the art. Typically the linker, for example a diamine linker,  
is asymmetrically protected (i.e. protected at one end) to form a water-soluble semi-  
15 protected diamine linker. The unprotected end of the linker, is reacted with a nucleotide  
which has been prepared by reacting the nucleotide with EDC and 1-methyl imidazole, as  
will be understood by one of ordinary skill in the art.

As already described herein, an alkyl-linked nucleotide attached to a solid support  
or a tag, such that the solid support or tag is suitable for the separation of the alkyl-linked  
20 nucleotide, and optionally, compounds (such as proteins, for example) bound to the alkyl-  
linked nucleotide, from unbound compounds, is also referred to herein as a "nucleotide  
affinity medium or media", or as an "alkyl-linked nucleotide affinity medium or media."

Generally, the linker is attached to the solid support in any suitable coupling  
buffer as will be understood by one of skill in the art. For example, the coupling buffer  
25 can be 0.1M or 0.2M sodium phosphate, pH adjusted to 8-9 for cyanogen bromide-  
activated SEPHAROSE™, or 0.1M or 0.2M sodium phosphate, pH adjusted to 10 for  
1,1'-carbonyl diimidazole-activated (CDI)- SEPHAROSE™. Alternatively, 0.01M to  
0.1M borate, with pH adjusted to 8-9 can be used for coupling cyanogen bromide-  
activated SEPHAROSE™, or 0.01M to 0.1M borate, with pH adjusted to 10 can be used  
30 for coupling CDI-activated SEPHAROSE™. For example, a linker can be reacted at

5 room temperature with cyanogen bromide-activated SEPHAROSE™ beaded agarose in a sodium bicarbonate coupling buffer (for example, and as used in the Examples below, 0.1 M NaHCO<sub>3</sub>, 0.5 M NaCl, pH = 8.2). For reactions with CDI-activated cross-linked SEPHAROSE™ beaded agarose, the linker can be, for example, reacted at room  
10 as will be understood by one of skill in the art. Typically, the solid support is then washed with water.

The solid support is then end-capped to block remaining active sites on the solid support and is a standard technique that can be performed with any suitable reagent, such as, ethanolamine, 2-amino-2-(hydroxymethyl)-1,3-propanediol, or glycine. To illustrate,  
15 end-capping can be achieved by reacting the solid support with 1M ethanolamine (pH = 8.9) for approximately 1 hour at room temperature. The solid support is then typically washed with 1M NaCl and water.

The nucleotide is then reacted with the linker arm on the solid support under suitable conditions, as will be understood by one of skill in the art, and as described in the  
20 Examples.

Once prepared, an alkyl-linked nucleotide bound to a solid support or tag (nucleotide affinity medium) is stored in any suitable buffer. For example, 0.1M K<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub> buffer (pH = 7.4), containing 0.02% sodium azide as a preservative.

Synthesis methods for isourea and carbamate linkages are standard in the art (see  
25 generally, Hermanson *et al.*, "Immobilized Affinity Ligand Techniques", Academic Press, 1992, the teaching of which is incorporated herein by reference in its entirety). For example, the use of a cyanogen bromide-activated agarose (see, generally, Cuatrecasas and Anfinsen, "Affinity Chromatography" in Ann. Rev. Biochem. Snell et al., eds. (CA: Annual Reviews Inc.), 40: 259-278 (1971), the teachings of which are incorporated  
30 herein by reference in their entirety) provides for the synthesis of a suitable isourea linkage, whereas the use of a CDI-activated cross-linked SEPHAROSE™ beaded agarose 6B (Pierce Biotechnology, Inc.) provides a suitable carbamate linkage. Alternatively, the hydroxyl group of a suitable resin can be converted into suitable leaving groups using N,N'-disuccinimidylcarbonate as an intermediate to prepare a carbamate linkage, or using

5 organic sulfonyl chlorides to activate the resin hydroxyl groups for nucleophilic displacement to prepare a carbon-nitrogen bond.

The alkyl-linked nucleotides bound to a solid support in the following exemplification were made with cyanogen bromide-activated SEPHAROSE™ beaded agarose 4B (Sigma), CDI-activated cross-linked SEPHAROSE™ beaded agarose 6B  
10 (Pierce Biotechnology, Inc.), TOYOPEARL® resins, SEPHACRYL™ resins, Trisacryl resins, or Ultrogel resins. Other suitable solid supports will be readily appreciated by one of skill in the art and include, for example and without limitation, acrylamide, agarose, methacrylate polymer, methacrylate copolymer, cellulose, nylon, silica, magnetized particle, nitrocellulose and polystyrene, and derivatives thereof.

15 Prior to use, cyanogen bromide-activated SEPHAROSE™ beaded agarose is washed with 1mM HCl and water, whereas CDI-activated cross-linked SEPHAROSE™ beaded agarose is washed with ice-cold water, in accordance with standard protocols, as will be understood by one of skill in the art.

One example of the chemical synthesis of a  $\gamma$ -phosphate-linked nucleotide affinity  
20 ligand is illustrated in Figs. 1-5. For the purpose of illustration, the nucleotide is ATP.

Figure 1 illustrates the chemical formation of Intermediate IA. Cyanogen bromide-activated SEPHAROSE™ beaded agarose is reacted with a diamino-hydrophobic linker to form a resin-bound linker (Intermediate IA).

Using an alternative resin, Figure 2 illustrates the chemical formation of  
25 Intermediate IB. CDI-activated SEPHAROSE™ beaded agarose is reacted with a diamino-hydrophobic linker to form a resin-bound linker (Intermediate IB).

The chemical formation of Intermediate II, which is the modification of the nucleotide in preparation of its attachment to the resin, is illustrated in Figure 3. The nucleotide is reacted with a water-soluble carbodiimide, such as 1-(3-  
30 dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC) to form O-phosphoryl isourea. The O-phosphoryl isourea is then reacted with a suitable nucleophilic compound, for example, 1-methyl imidazole to form Intermediate II.

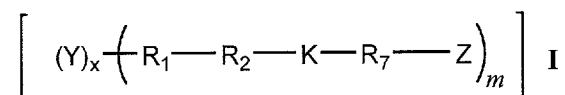
The final steps for the synthesis of nucleotide affinity media are illustrated in Figures 4 and 5. Specifically, Fig. 4 illustrates one example of the chemical formation of  
35 a nucleotide affinity medium. Intermediates IA and II are combined to form the final

5 alkyl-linked nucleotide bound to a solid support. In another example, shown in Figure 5, Intermediates IB and II are combined to form the final alkyl-linked nucleotide bound to a solid support.

In one embodiment, the loading of a solid support with an alkyl-linked nucleotide can be varied. This means that not necessarily all reactive sites on a solid support are  
10 reacted with an alkyl-linked nucleotide. For example, the loading of a solid support with an alkyl-linked nucleotide can be in a range of 5-25%, meaning 5-25% of reactive sites are reacted with an alkyl-linked nucleotide. Alternatively, the loading of the alkyl-linked nucleotide is in a range of 20-50%, 40-65%, 60-80% or 75-100%. The reactive groups on the solid support which are not reacted with an alkyl-linked nucleotide can be capped  
15 using a suitable reagent as appropriate.

### Utility of Alkyl-linked Nucleotide Compositions

Also included in the invention is a method for screening compounds, for example, with a proteome comprising the steps of (a) contacting a proteome with a nucleotide  
20 affinity medium comprising a general formula:



wherein Y is a solid support or a tag;  $x = 1$ ;  $R_1$  is a covalent bond between Y and  $R_2$ , or  $R_1$  is an acyl group, a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a  
25 substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof;  $R_2$  is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a  
30 combination thereof; K is a heteroatom;  $R_7$  is  $(P)_n$  where P is a phosphate or thiophosphate and n is at least one or  $R_7$  is a phosphate group mimic, Z is a nucleoside or nucleoside derivative; and m is at least one; (b) washing the nucleotide affinity medium with a buffer, whereby non-specifically bound components of the proteome are eluted from the nucleotide affinity medium and specific components of the proteome remain

5 bound to the nucleotide affinity medium; (c) contacting the nucleotide affinity medium  
bound to specific components of the proteome with at least one test compound; (d)  
eluting from the nucleotide affinity medium components of the proteome that are  
specifically displaced by the test compound; and (e) identifying the components of the  
proteome that are specifically displaced by the test compound from the nucleotide affinity  
10 medium.

A test compound can be any compound that is organic or inorganic, naturally-  
occurring or non-naturally occurring, as will be appreciated by one of skill in the art. For  
example, the test compound can be a compound from a combinatorial library or a  
chemical library. Furthermore, the test compound can be a compound extracted from a  
15 single cellular organism, a multicellular organism, or from an organ or a tissue of a  
multicellular organism. Examples of such organisms include, without limitation,  
bacteria, algae, fungi, plant, fish, amphibians, mammals, and the like. The test compound  
can be a single compound, or alternatively, a mixture of compounds, as will be  
understood by one of skill in the art.

20 As described elsewhere herein, a solid support can be any suitable support, such  
as a resin, or a particulate material, such as a bead, or a particle. Alternatively, a solid  
support can be a continuous solid surface, such as a plate, chip, well, channel, column or  
a tube. The material of a solid support will be of any suitable substance, compound or  
polymer, as will be appreciated by one of skill in the art. Examples include, without  
25 limitation, acrylamide, agarose, methacrylate polymer, methacrylate copolymer,  
cellulose, nylon, silica, glass, ceramic, a magnetized particle or surface, nitrocellulose,  
polystyrene and derivatives thereof.

Furthermore, a "tag", as that term is employed herein, is an agent that provides for  
the specific detection or capture of the alkyl-linked nucleotide. For example, and without  
30 limitation, a suitable tag is biotin, avidin, streptavidin, a hapten, a fluorophore or a  
chromophore. Detection or capture of the alkyl-linked nucleotide employs techniques  
that are standard in the art. For example, a biotin-tagged alkyl-linked nucleotide can be  
captured using avidin, streptavidin or related avidin derivatives. The biotin-avidin  
interaction is highly specific. An avidin-conjugated agent used to detect or capture a  
35 biotin-tagged alkyl-linked nucleotide can be soluble (for example, an antibody), or a

5 particulate material (for example, beaded agarose or a magnetized particle), or a continuous surface (for example, a plate or well coated with avidin). Detection and capture of a hapten-tagged alkyl-linked nucleotide can be achieved, for example, using hapten-specific antibodies. Visual detection methods for fluorophore or chromophore-tagged alkyl-linked nucleotides are readily understood by one of skill in the art. The  
10 visual detection of fluorophore or chromophore-tagged alkyl-linked nucleotides will be useful for techniques such as rapidly determining the presence or absence of a specific interaction between the tagged alkyl-linked nucleotide and a target protein. Alternatively, the visual detection of fluorophore or chromophore-tagged alkyl-linked nucleotides will be useful for detecting the presence or absence of a specific interaction  
15 between a test compound and a tagged alkyl-linked nucleotide bound to a target protein. Furthermore, linker-specific effects on the affinity or avidity of the affinity ligand for a compound can be monitored using fluorophore or chromophore-tagged alkyl-linked nucleotides. For example, a linker can affect the selectivity, affinity or avidity of the alkyl-linked nucleotide for an interacting compound via steric hindrance or electrostatic  
20 interactions, for example. These linker-specific effects can be assayed or monitored by visually detecting which linkers attached to a nucleotide specifically affect the interaction of the nucleotide with the target protein or compound. Such linkers can be used to prepare nucleotide affinity media that selectively bind a subset of proteins or target compounds.

25 As described elsewhere herein, a nucleoside and a nucleotide, as referred to herein, can be naturally-occurring or non-naturally-occurring. Furthermore, the nucleoside or nucleotide can be biologically or synthetically-derived using techniques that are standard to one of skill in the art. Suitable nucleosides include, without limitation, adenosine (A), guanosine (G), cytidine (C), thymidine (T) and uridine (U).

30 In one embodiment, the nucleotide is a monophosphate, diphosphate, or triphosphate of adenosine, guanosine, cytidine, thymidine, or uridine or an analog thereof.

The alkyl-linked nucleotide affinity media of the invention can be used, for example, in affinity chromatography techniques using methods that are known to one of  
35 skill in the art. See, for example, WO 00/63694, filed 12 April, 2000 and U.S. Patent No.

5 5,536,822, filed March 4, 1994, the teachings of which are incorporated herein by reference in their entirety.

The nucleotide affinity media and alkyl-linked nucleotides are useful for the detection and purification of biological compounds that bind to a nucleotide. For example,  $\gamma$ -phosphate-linked adenosine triphosphate (ATP) can be used to detect and  
10 purify biological compounds such as kinases, which are known to bind ATP. Specifically, an alkyl-linked nucleotide, such as ATP, can be bound to a solid support or tag, and subsequently contacted with, or mixed with, a proteome or part thereof. Non-specifically-interacting components of a proteome are generally removed by washing with a suitable buffer, as will be readily understood by one of skill in the art.

15 Additionally, the alkyl-linked nucleotide can be used to screen chemical compounds that specifically interact with a protein captured by the alkyl-linked nucleotide. Subsequently, this protein can be identified using art-standard techniques.

Alternatively, the alkyl-linked nucleotide can be used to detect and isolate chemical compounds that specifically bind to the nucleotide. For example, an alkyl-  
20 linked nucleotide can be mixed with a combinatorial library. Compounds of the combinatorial library that specifically interact with the alkyl-linked nucleotide can be separated from non-specifically-interacting compounds, for example, by washing with one or more suitable buffers. Subsequently, the specifically-interacting compounds can be identified using art-standard techniques.

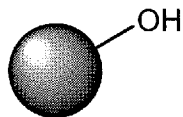
25 Alternatively, competitors of compounds which are known to interact with a nucleotide can be identified. For example, an alkyl-linked nucleotide can be mixed with a known interacting compound, such as a protein, to allow for their binding to occur. Then another compound or library of compounds (such as a combinatorial library) can be added to the alkyl-linked nucleotide bound to the known compound. If one or more  
30 different compounds can compete for binding to the nucleotide (and thus, displace the first bound compound), the known compound will be released and the competitor compound can be subsequently identified. Such compounds may be useful biological or pharmacological inhibitors.



5

## EXEMPLIFICATION

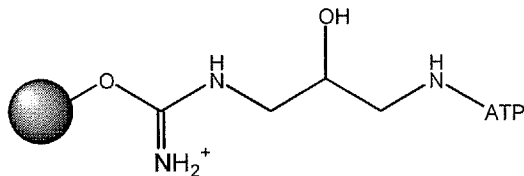
The present invention is further illustrated by the following examples, which are not intended to be limiting in any way. For the purpose of simplicity of illustration, the solid support is generically represented as:



10 wherein, generally a hydroxyl group on the solid support is available to react with a suitable reagent, for example cyanogen bromide which will form a cyanogen bromide-activated solid support. The solid support may be any suitable solid support as described above.

### 15 EXAMPLE 1

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 1:



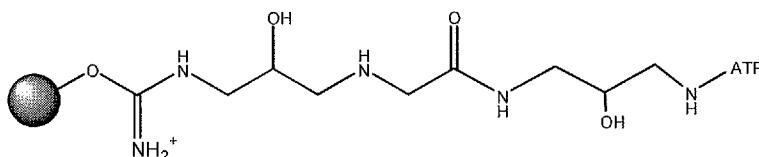
20 The linker arm was attached to the resin by adding 1g of 1,3-diamino-2-propanol to 30mL coupling buffer and combining with 2g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 3 hours at room temperature.

The affinity ligand, ATP, was prepared for attachment to the resin by adding 500  
25 mg ATP, 103.5mg N-hydroxysuccinimide and 172.5mg EDC to 20mL water, and reacting for approximately 2 hours at room temperature.

Finally, the nucleotide affinity media was prepared by adding 111mg 4-dimethylaminopyridine to the activated affinity ligand and combining with the prepared resin and allowing the reaction to continue for approximately 12-18 hours, or overnight,  
30 at room temperature.

## 5 EXAMPLE 2

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 2:



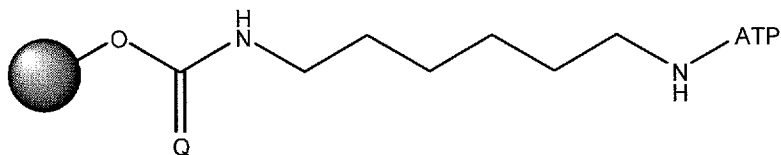
10 The linker was attached to the resin by first adding 1g 1,3-diamino-2-propanol to 30mL coupling buffer and combining with 2g cyanogen bromide-activated SEPHAROSE™. The reaction proceeded for approximately 3 hours. A second reaction of the resin was performed by adding 1.5g iodoacetic acid to 30mL coupling buffer, and adjusting the pH to 9.8 and combining with the resin. This was reacted for a further 1  
15 hour, approximately, at room temperature. In a further reaction with the resin, 500 mg EDC was added to 20mL water and combined with the resin. Reaction proceeded for a further 30 minutes. Subsequently, 1g 1,3-diamino-2-propanol was added to 30 mL coupling buffer and combined with the resin. Reaction continued for another 1 hour, approximately.

20 The affinity ligand was prepared by adding 500 mg ATP, 103.5mg N-hydroxysuccinimide and 172.5mg EDC to 20mL water, and allowing the reaction to proceed for approximately 2 hours.

Finally, 111mg 4-dimethylaminopyridine was added to the prepared affinity ligand and combined with resin. Reaction was allowed to proceed for approximately 12-  
25 18 hours, or overnight at room temperature.

## EXAMPLES 3-8

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples  
30 3-8:



5        wherein Q =  $\text{NH}_2^+$  or O

### EXAMPLE 3

To prepare the resin, 250 mg 1,6-diaminohexane was added to 5mL coupling buffer, combined with 500mg cyanogen bromide-activated SEPHAROSE™ and reacted  
10 for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 300 mg ATP, 104mg EDC and 62mg N-hydroxysuccinimide to 7mL water, reacting for 90 minutes at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

15

### EXAMPLE 4

To prepare the resin, 250mg 1,6-diaminohexane was added to 5mL coupling buffer and combined with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction proceeded for approximately 2 hours at room temperature.

20        The affinity ligand was prepared by adding 690mg ATP, 513μL 1-methyl imidazole and 1200mg EDC to 6mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

25

### EXAMPLE 5

To prepare the resin, 750mg 1,6-diaminohexane was added to 7.5mL coupling buffer and combined with 1.425g cyanogen bromide-activated SEPHAROSE™. The reaction proceeded for approximately 2 hours at room temperature.

5           The affinity ligand was prepared by adding 1378mg ATP, 1027μL 1-methyl  
imidazole and 2400mg EDC to 6mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 6

          To prepare the resin, 225mg 1,6-diaminohexane was added to 1.5mL coupling  
buffer and combine with 285mg cyanogen bromide-activated SEPHAROSE™. The  
reaction proceeded for approximately 2 hours at room temperature.

15           The affinity ligand was prepared by adding 550 mg ATP, 410μL 1-methyl  
imidazole and 960mg EDC to 5mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

20

#### EXAMPLE 7

          To prepare the resin, 150 mg 1,6-diaminohexane was added to 3mL coupling  
buffer, the pH was adjusted to pH 8.4, and then combined with 571mg cyanogen bromide  
activated SEPHAROSE™. The reaction proceeded for approximately 2 hours at room  
25   temperature.

          The affinity ligand was prepared by adding 1100mg ATP, 820μL 1-methyl  
imidazole and 1920mg EDC to 10mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and affinity ligand were then combined together and allowed to react  
30   for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 8

          To prepare the resin, 250mg 1,6-diaminohexane was added to 5mL coupling  
buffer and combines with 2.5mL CDI-activated cross-linked SEPHAROSE™. The  
35   reaction proceeded for approximately 2 hours at room temperature.

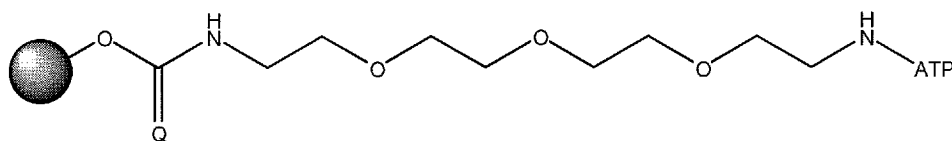
5           The affinity ligand was prepared by adding 690mg ATP, 513μL 1-methyl  
imidazole and 1200mg EDC to 6mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLES 9-28

          The following general structure represents the affinity ligand bound to a solid  
support when synthesized using the linker and reaction conditions described in Examples  
9-28:



15

wherein Q =  $\text{NH}_2^+$  or O

For all Examples 9-16, the resin was prepared by adding 700 μL 1,11-diamino-3,6,9-  
trioxaundecane to 10mL coupling buffer and combining with 2g cyanogen bromide-  
activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2  
hours at room temperature. Aliquots of 300 mg of the reacted resin were used for

20

Examples 9-16.

#### EXAMPLE 9

          The affinity ligand was prepared by adding 275mg ATP, 58mg N-  
hydroxysuccinimide and 96mg EDC to 3mL water, and reacting for approximately 2  
25 hours at room temperature.

          The affinity ligand and 300mg of prepared resin (above) were combined and  
allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 10

30           The affinity ligand was prepared by adding 275mg ATP, 41μL 1-methyl  
imidazole and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room  
temperature.

- 5           The affinity ligand and 300mg of prepared resin (above) were combined and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 11

- 10           The affinity ligand was prepared by adding 275mg ATP, 290mg N-hydroxysuccinimide and 480mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand and 300mg of prepared resin (above) were combined and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### 15   EXAMPLE 12

          The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methylimidazole and 480mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

- 20           The affinity ligand and 300mg of prepared resin (above) were combined and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 13

- 25           The affinity ligand was prepared by adding 275mg ATP, 58mg N-hydroxysuccinimide and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 5 hours at room temperature.

- 30           Alternatively, the affinity ligand was prepared by adding 275mg ATP, 58mg N-hydroxysuccinimide and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5     EXAMPLE 14

          The affinity ligand was prepared by adding 275mg ATP, 41μL 1-methyl imidazole and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for  
10    approximately 5 hours at room temperature.

          Alternatively, the affinity ligand was prepared by adding 275mg ATP, 41μL 1-methyl imidazole and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for  
15    approximately 12-18 hours, or overnight at room temperature.

          EXAMPLE 15

          The affinity ligand was prepared by adding 275mg ATP, 290mg N-hydroxysuccinimide and 480mg EDC to 3mL water, and reacting for approximately 2  
20    hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 5 hours at room temperature.

          Alternatively, the affinity ligand was prepared by adding 275mg ATP, 290mg N-hydroxysuccinimide and 480mg EDC to 3mL water, and reacting for approximately 2  
25    hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 12-18 hours, or overnight at room temperature.

          EXAMPLE 16

30        The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 5 hours at room temperature.

5           Alternatively, the affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 17

          The resin was prepared by adding 500μL 1,11-diamino-3,6,9-trioxaundecane to 10mL coupling buffer and combining with 2mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at  
15 room temperature.

          The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 8mL water, and reacting for approximately 1 hour at room temperature.

          The affinity ligand was combined with the prepared resin and allowed to react for  
20 approximately 12-18 hours, or overnight at room temperature.

          Alternatively, the affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

          The affinity ligand was combined with the prepared resin and allowed to react for  
25 approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 18

          The resin was prepared by adding 200μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked  
30 SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.



5           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature..

#### EXAMPLE 19

10           The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

15           The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 20

20           The resin was prepared by adding 250μL 1,11-diamino-3,6,9-trioxaundecane to 5mL coupling buffer and combining with 2.5mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25           The affinity ligand was prepared by adding 275mg ATP, 82μL 1-methyl imidazole and 192mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

30           Alternatively, the affinity ligand was prepared by adding 250mg ATP, 195μL 1-methyl imidazole and 430mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5     EXAMPLE 21

The resin was prepared by adding 75 $\mu$ L ethanolamine and 75 $\mu$ L 1,11-diamino-3,6,9-trioxaundecane to 3mL coupling buffer and combining with 1.5mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

10     The affinity ligand was prepared by adding 415mg ATP, 125 $\mu$ L 1-methyl imidazole and 290mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

15

EXAMPLE 22

The resin was prepared by adding 135 $\mu$ L ethanolamine and 15 $\mu$ L 1,11-diamino-3,6,9-trioxaundecane to 3mL coupling buffer and combining with 1.5mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

20

The affinity ligand was prepared by adding 415mg ATP, 125 $\mu$ L 1-methyl imidazole and 290mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

25

EXAMPLE 23

The resin was prepared by adding 142.5 $\mu$ L ethanolamine and 7.5 $\mu$ L 1,11-diamino-3,6,9-trioxaundecane to 3mL coupling buffer and combining with 1.5mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

30

The affinity ligand was prepared by adding 415mg ATP, 125 $\mu$ L 1-methyl imidazole and 290mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

5           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 24

10           The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked SEPHAROSE™. The reaction was reacted for approximately 2 hours at room temperature.

15           The affinity ligand was prepared by adding 275mg ATP, 82μL 1-methyl imidazole and 192mg EDC to 3mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature..

#### EXAMPLE 25

20           The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25           The affinity ligand was prepared by adding 138mg ATP, 103μL 1-methyl imidazole and 240mg EDC to 3mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### 30   EXAMPLE 26

          The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

5           The affinity ligand was prepared by adding 138mg ATP, 41μL 1-methyl  
imidazole and 96mg EDC to 3mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 27

          The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to  
2mL coupling buffer and combining with 1mL CDI-activated cross-linked  
SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at  
15 room temperature.

          The affinity ligand was prepared by adding 275mg ATP, 204μL 1-methyl  
imidazole and 96mg EDC to 3mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
20 for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 28

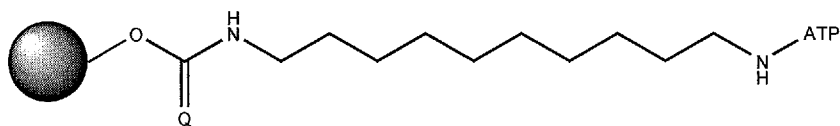
          The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to  
2mL coupling buffer and combining with 1mL CDI-activated cross-linked  
25 SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at  
room temperature.

          The affinity ligand was prepared by adding 275mg ATP, 41μL 1-methyl  
imidazole and 480mg EDC to 3mL water, and reacting for approximately 1 hour at room  
temperature.

30           The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

5   EXAMPLES 29-36

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 29-36:



10   wherein Q =  $\text{NH}_2^+$  or O

EXAMPLE 29

The resin was prepared by adding 250 mg 1,10-diaminodecane and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 571 mg cyanogen bromide-activated  
15   SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 550 mg ATP, 410  $\mu\text{L}$  1-methyl imidazole and 960 mg EDC to 6 mL water, and reacting for approximately 1 hour at room temperature.

20   The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

EXAMPLE 30

The resin was prepared by adding 125mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated  
25   SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 482 mg ATP, 359  $\mu\text{L}$  1-methyl imidazole and 840 mg EDC to 5.25 mL water, and reacting for approximately 1 hour at  
30   room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5    EXAMPLE 31

The resin was prepared by adding 250mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

10    The affinity ligand was prepared by adding 482mg ATP, 359μL 1-methyl imidazole and 840mg EDC to 5.25mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

15

EXAMPLE 32

The resin was prepared by adding 219mg 1,10-diaminodecane and 0.875mL 1,4-dioxane to 3.5mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2  
20 hours at room temperature.

The affinity ligand was prepared by adding 482mg ATP, 359μL 1-methyl imidazole and 840mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

25    The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

EXAMPLE 33

30    The resin was prepared by adding 219mg 1,10-diaminodecane and 0.875mL 1,4-dioxane to 3.5mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

5           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 34

10           The resin was prepared by adding 125mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

15           The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methylimidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 35

20           The resin was prepared by adding 250 mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25           The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methylimidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### 30   EXAMPLE 36

          The resin was prepared by adding 125mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

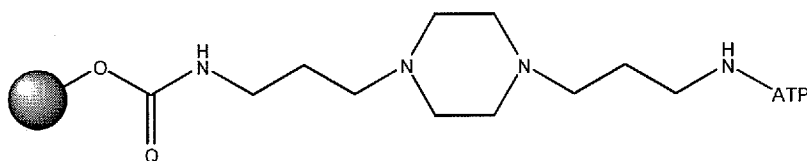
5           The affinity ligand was prepared by adding 482mg ATP, 359μL 1-methyl imidazole and 840mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 37

          The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 37:



15

wherein Q = NH<sub>2</sub><sup>+</sup> or O

          The resin was prepared by adding 125μL 1,4-bis(3-aminopropyl)piperazine and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

20

          The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

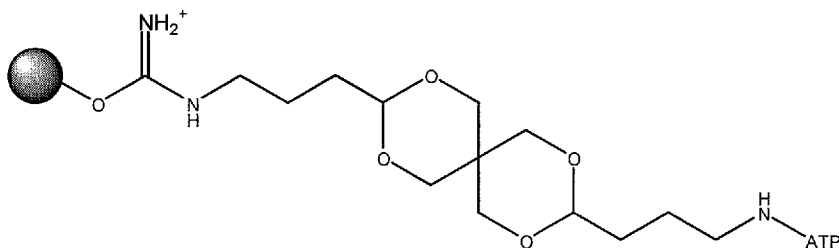
          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

25

#### EXAMPLE 38

          The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 38:





5

The resin was prepared by adding 125mg 2,4,8,10-tetraoxaspiro[5.5]undecane-3,9-dipropanamine and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

10

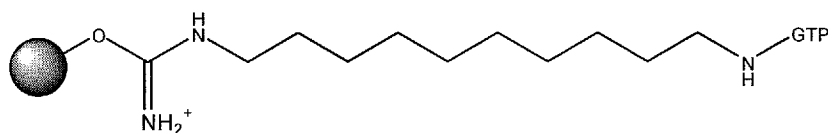
The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methyl imidazole and 40mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

15

#### EXAMPLE 39

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 39:



20

The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25

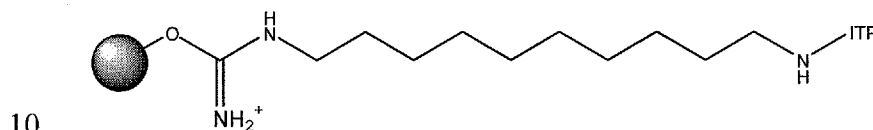
The affinity ligand was prepared by adding 39mg GTP, 31μL 1-methyl imidazole and 72mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5

## EXAMPLE 40

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 40:



The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

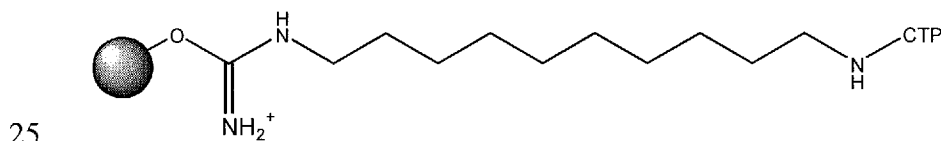
15 The affinity ligand was prepared by adding 43mg ITP, 31μL 1-methyl imidazole and 72mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

20

## EXAMPLE 41

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 41:



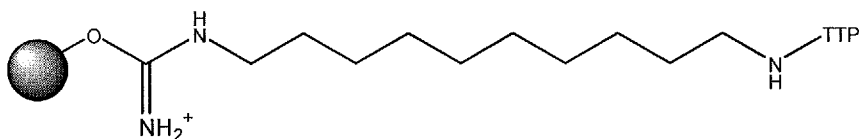
The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

30 The affinity ligand was prepared by adding 40mg CTP, 31μm 1-methyl imidazole and 72mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

5           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 42

10           The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 42:



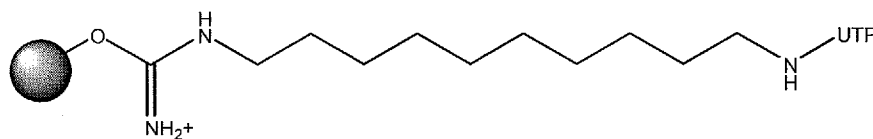
15           The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 36 mg TTP, 31μL 1-methyl imidazole and 72 mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

20           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 43

25           The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 43:



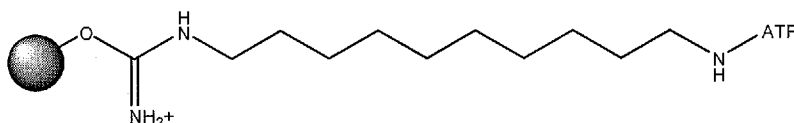
30           The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

5 The affinity ligand was prepared by adding 37mg UTP, 31μL 1-methyl imidazole and 72 mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature. React overnight.

#### 10 EXAMPLES 44-48

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 44-48:



#### EXAMPLE 44

20 The resin was prepared by adding 187.5mg 1,10-diaminodecane, 62.5μL ethanolamine and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature..

25 The affinity ligand was prepared by adding 480mg ATP, 360μL 1-methyl imidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 45

30 The resin was prepared by adding 125mg 1,10-diaminodecane, 125μL ethanolamine and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

5           The affinity ligand was prepared by adding 480mg ATP, 360μL 1-methyl  
imidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 46

          The resin was prepared by adding 62.5mg 1,10-diaminodecane, 187.5μL  
ethanolamine and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g  
cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for  
15 approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 480mg ATP, 360μL 1-methyl  
imidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
20 for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 47

          The resin was prepared by adding 25mg 1,10-diaminodecane, 225μL  
ethanolamine and 1mL, 1,4-dioxane to 4mL coupling buffer and combining with 1g  
25 cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for  
approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 480 mg ATP, 360 μL 1-methyl  
imidazole and 840 mg EDC to 5 mL water, and reacting for approximately 1 hour at  
room temperature.

30           The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 48

          The resin was prepared by adding 83 mg 1,10-diaminodecane, 167 μL  
35 ethanolamine and 1 mL 1,4-dioxane to 4 mL coupling buffer and combining with 1 g

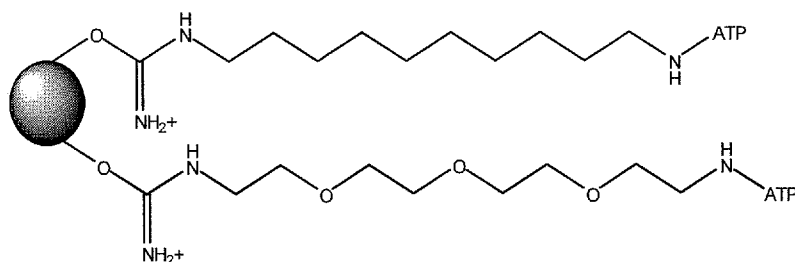
5 cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 480 mg ATP, 360  $\mu$ L 1-methyl imidazole and 840 mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

10 The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLES 49-50

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linkers and reaction conditions described in  
15 Examples 49-50:



#### EXAMPLE 49

20 The resin was prepared by adding 125mg 1,10-diaminodecane, 125 $\mu$ L 1,11-diamino-3,6,9-trioxaundecane and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 480mg ATP, 360 $\mu$ L 1-methyl  
25 imidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5     EXAMPLE 50

The resin was prepared by adding 62.5mg 1,10-diaminodecane, 187.5μL 1,11-diamino-3,6,9-trioxaundecane and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

10     The affinity ligand was prepared by adding 480mg ATP, 360μL 1-methyl imidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

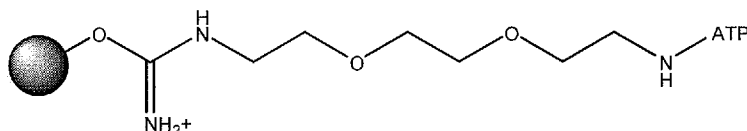
The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

15

EXAMPLE 51

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 51:

20



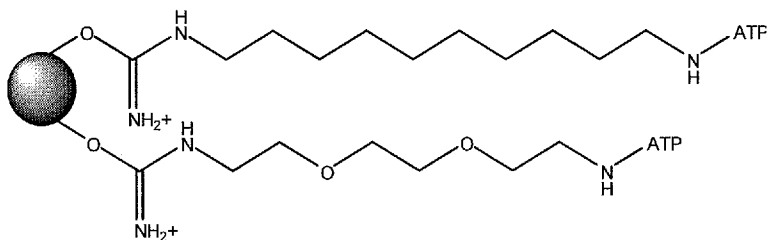
25     The resin was prepared by adding 125μL 1,8-diamino-3,6-dioxaoctane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

30     The affinity ligand was prepared by adding 240mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

## 5 EXAMPLES 52-53

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linkers and reaction conditions described in Examples 52-53:



10

### EXAMPLE 52

The resin was prepared by adding 62.5mg 1,10-diaminodecane, 62.5μL 1,8-diamino-3,6-dioxaoctane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

15

The affinity ligand was prepared by adding 240mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

20

### EXAMPLE 53

The resin was prepared by adding 31.5mg 1,10-diaminodecane, 93.75μL 1,8-diamino-3,6-dioxaoctane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25

The affinity ligand was prepared by adding 240mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

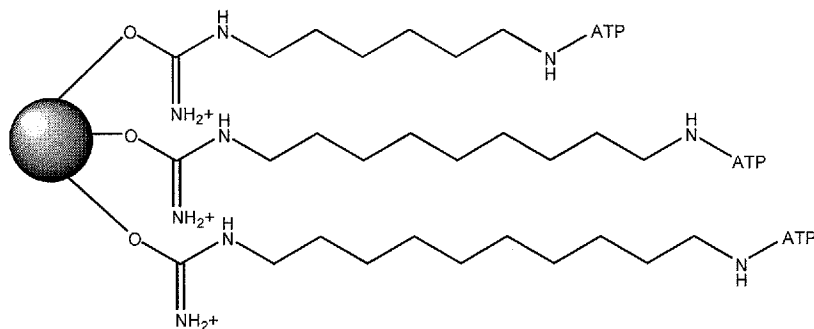
30



5

## EXAMPLES 54-56

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linkers and reaction conditions described in Examples 54-56:



10

For Examples 54-56, a mixture of 100mg of each linker arm: 1,10-diaminodecane; 1,9-diaminononane; and 1,6-diaminohexane, was prepared for use in the preparation of the nucleotide affinity media described.

## 15 EXAMPLE 54

The resin was prepared by adding 125mg of the mixture of linker arms (above), and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

20 The affinity ligand was prepared by adding 240mg ATP, 180  $\mu$ L 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

25

## EXAMPLE 55

The resin was prepared by adding 62.5 mg of the mixture of linker arms (above), 62.5  $\mu$ L ethanolamine and 0.5mL 1,4-dioxane to 2 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

30

5           The affinity ligand was prepared by adding 240 mg ATP, 180  $\mu$ L 1-methyl  
imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

#### 10       EXAMPLE 56

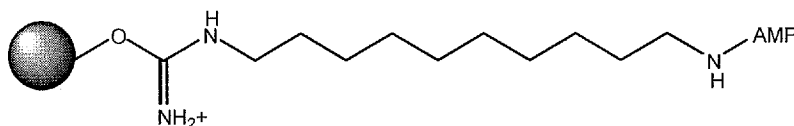
          The resin was prepared by adding 31.25 mg of the mixture of linker arms (above),  
93.75  $\mu$ L ethanolamine and 0.5 mL 1,4-dioxane to 2 mL coupling buffer and combining  
with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to  
15       proceed for approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 240 mg ATP, 180  $\mu$ L 1-methyl  
imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and affinity ligand were then combined together and allowed to react  
20       for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 57

          The following general structure represents the affinity ligand bound to a solid  
support when synthesized using the linker and reaction conditions described in Example  
25       57:



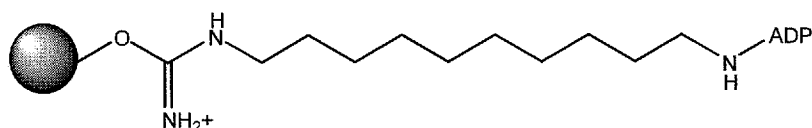
          The resin was prepared by adding 125 mg 1,10-diaminodecane and 0.5 mL 1,4-  
dioxane to 2 mL coupling buffer and combining with 500 mg cyanogen bromide-  
activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2  
30       hours at room temperature.

          The affinity ligand was prepared by adding 160mg AMP, 180  $\mu$ L 1-methyl  
imidazole and 420 mg EDC to 2.5mL water, and reacting for approximately 1 hour at  
room temperature.

5           The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 58

10           The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 58:



15           The resin was prepared by adding 125 mg 1,10-diaminodecane and 0.5 mL 1,4-dioxane to 2 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 188 mg ADP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

20           The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

General procedure for SEPHACRYL™ and TOYOPEARL® resins:

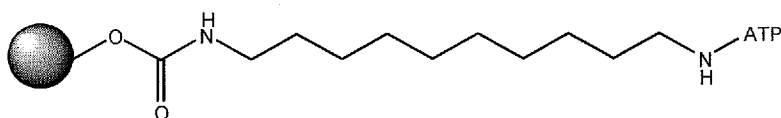
25           SEPHACRYL™ and TOYOPEARL® resins are generally stored in 20% ethanol. After washing 2.5 mL of the resin with 2 volumes of water, 1 volume of 30% acetone, 1 volume of 70% acetone and 5 volumes of dry acetone the resin is transferred into 2.5 mL dry acetone. 200 mg 1,1'-carbonyl diimidazole is added and the resin is allowed to react for approximately 1 hour at room temperature. After approximately 1 hour the resin is washed with 5 volumes of dry acetone, 1 volume of 70% acetone, 1 volume of 30% acetone and 2 volumes of water. 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of a suitable coupling buffer and combined with the resin. The resin is allowed to react overnight at 4°C. The resin is then washed with three volumes of 1 M NaCl and two volumes of water. To end-cap any remaining reactive sites, 5 mL of a 1 M

30

5 ethanolamine solution (pH = 8.9) is added to the resin and the resin is allowed to react at room temperature for approximately 1 hour. The resin is subsequently washed with three volumes of 1 M NaCl and two volumes of water. 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC are added to 10 mL water, and after reacting for approximately 1 hour, are combined with the resin. The resin is allowed to react  
10 overnight. Finally the resin is washed with three volumes of 1 M NaCl and two volumes of water and transferred into a storage buffer.

#### EXAMPLES 59-64

The following general structure represents the affinity ligand bound to a solid  
15 support when synthesized using the linker and reaction conditions described in Examples 59-64:



#### EXAMPLE 59

20 The resin is prepared by combining 2.5 mL of HR S-300 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any  
25 remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

The affinity ligand is prepared by adding 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and  
30 affinity ligand are reacted for approximately 12-18 hours, or overnight.

5   EXAMPLE 60

The resin is prepared by combining 2.5 mL of HR S-300 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined  
10 with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and  
15 affinity ligand are reacted for approximately 12-18 hours, or overnight.

EXAMPLE 61

The resin is prepared by combining 2.5 mL of HR S-300 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed  
20 to proceed for approximately 1 hour. Next, 62.5 mg 1,10-diaminodecane, 187.5 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl  
25 imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

EXAMPLE 62

30       The resin is prepared by combining 2.5 mL of HR S-400 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any

5 remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

The affinity ligand is prepared by adding 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and  
10 affinity ligand are reacted for approximately 12-18 hours, or overnight.

#### EXAMPLE 63

The resin is prepared by combining 2.5mL of HR S-400 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed  
15 to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combine with resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720  $\mu$ L 1-methyl  
20 imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

#### EXAMPLE 64

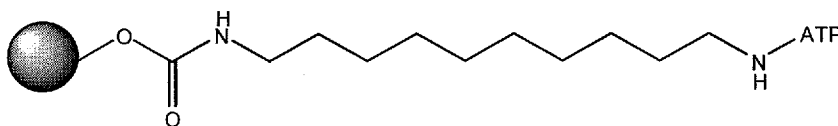
25 The resin is prepared by combining 2.5 mL of HR S-400 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 62.5mg 1,10-diaminodecane, 187.5 $\mu$ L ethanolamine and 1mL 1,4-dioxane are added to 4mL of coupling buffer and combine with resin. The reaction is allowed to proceed for approximately 12-18 hours, or  
30 overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

35

5   EXAMPLES 65-70

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 65-70:



10

EXAMPLE 65

The resin is prepared by combining 2.5 mL of HW-65F TOYOPEARL® resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next 250 mg 1,10-diaminodecane and 1mL 1,4-dioxane are added to 4mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

EXAMPLE 66

The resin is prepared by combining 2.5 mL of HW-65F TOYOPEARL® resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for

5 approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

#### EXAMPLE 67

The resin is prepared by combining 2.5 mL of HW-65S TOYOPEARL® resin  
10 with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution  
15 to the resin and reacting for approximately 1 hour.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

20

#### EXAMPLE 68

The resin is prepared by combining 2.5 mL of HW-65S TOYOPEARL® resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed  
25 to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl  
30 imidazole and 1680 mg EDC to 10mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.



5    EXAMPLE 69

          The resin is prepared by combining 2.5 mL of HW-75F TOYOPEARL® resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction  
10    is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

          The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for  
15    approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

EXAMPLE 70

          The resin is prepared by combining 2.5mL of HW-75F TOYOPEARL® resin  
20    with 2.5mL dry acetone and 200mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

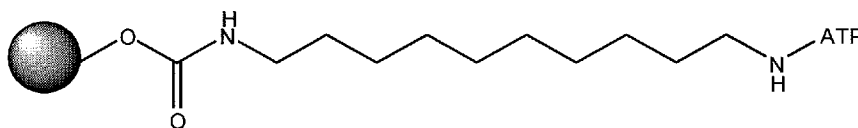
25          The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

30          The procedure for the AF-Tresyl-650M TOYOPEARL® resin is different than for the other TOYOPEARL® resins, since this resin comes with its hydroxyl functionalities pre-activated with tresyl chloride (an organic sulfonyl chloride), similar to cyanogen bromide-activated SEPHAROSE™ or 1,1'-carbonyl diimidazole-activated SEPHAROSE™.

35

5   EXAMPLES 71-72

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 71-72:



10

EXAMPLE 71

The resin is prepared by allowing 0.5 g of AF-Tresyl-650M TOYOPEARL® resin to swell in 15 mL 1mM HCl for approximately 10 minutes. The resin is then washed with 2 volumes of 1mM HCl and 1 volume of water. Next, 250 mg 1,10-  
15   diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer (0.2 M sodium phosphate buffer, pH = 7.45) and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4° C. Any remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

20       The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

25   EXAMPLE 72

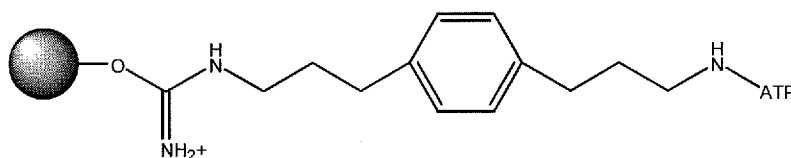
The resin is prepared by allowing 0.5 g of AF-Tresyl-650M TOYOPEARL® resin to swell in 15 mL HCl for approximately 10 minutes. The resin is then washed with 2 volumes of 1 mM HCl and 1 volume of water. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1mL 1,4-dioxane are added to 4mL of coupling buffer (0.2M  
30   sodium phosphate buffer, pH = 7.45) and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10mL water. The reaction is allowed to proceed for

- 5 approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

#### EXAMPLE 73

- 10 The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 73:



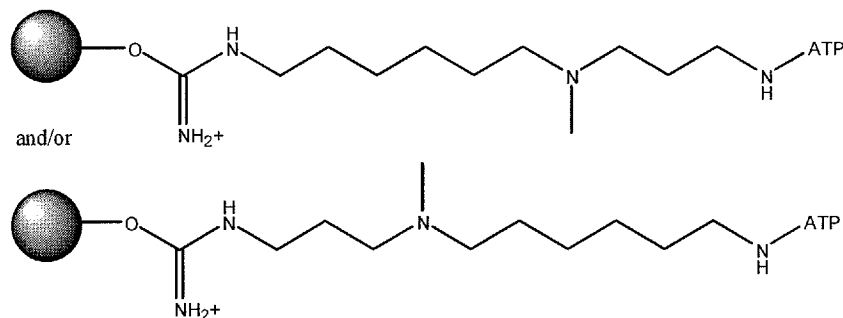
- 15 The resin was prepared by adding 190 mg 3-[4-(3-aminopropyl)-phenyl]-propylamine and 1.5 mL 1,4-dioxane to 3 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 5 hours at room temperature.

The affinity ligand was prepared by adding 480 mg ATP, 360  $\mu\text{L}$  1-methylimidazole and 840 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

- 20 The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 74

- 25 The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 74:



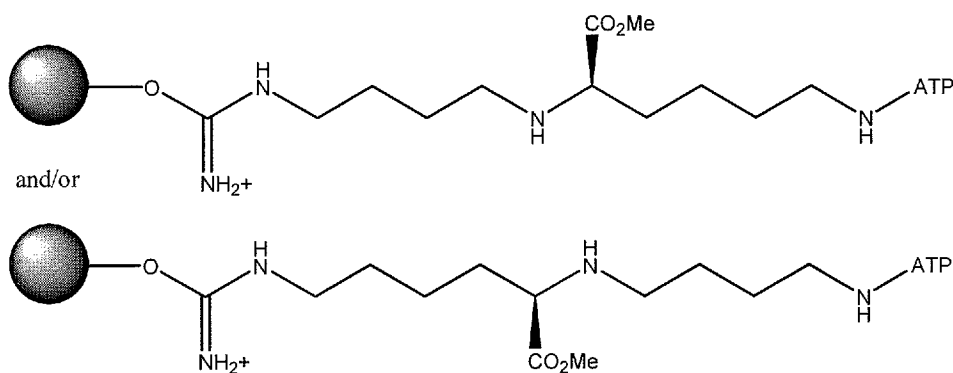
5        The resin was prepared by adding 190 mg N-(3-aminopropyl)-N-methyl-hexane-1,6-diamine and 0.75 mL 1,4-dioxane to 3 mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 5 hours at room temperature.

10       The affinity ligand was prepared by adding 480 mg ATP, 360μL 1-methylimidazole and 840 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

      The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

## 15    EXAMPLE 75

      The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 75:



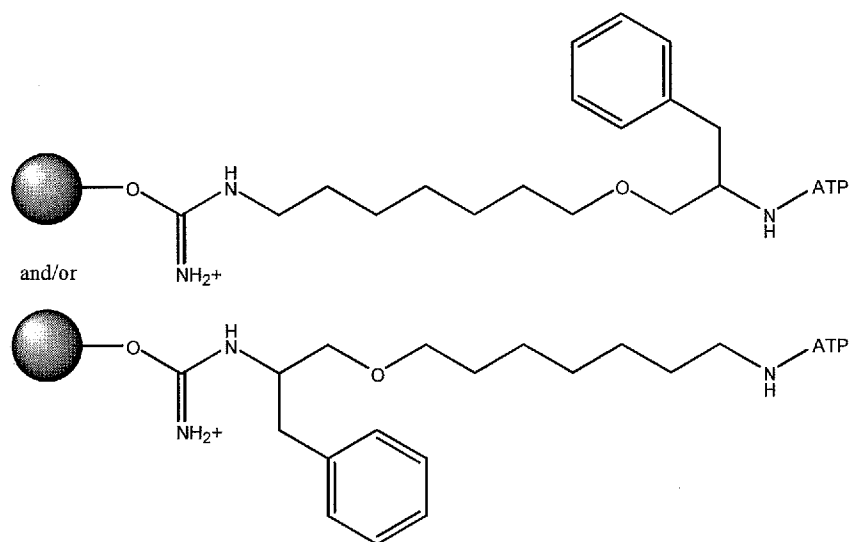
20       The resin was prepared by adding 350 mg 6-amino-2-(4-amino-butylamino)-hexanoic acid methyl ester and 1.4 mL 1,4-dioxane to 5.6 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 5 hours at room temperature.

25       The affinity ligand was prepared by adding 480 mg ATP, 360 μL 1-methylimidazole and 840 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

      The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

## 5 EXAMPLE 76

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 76:



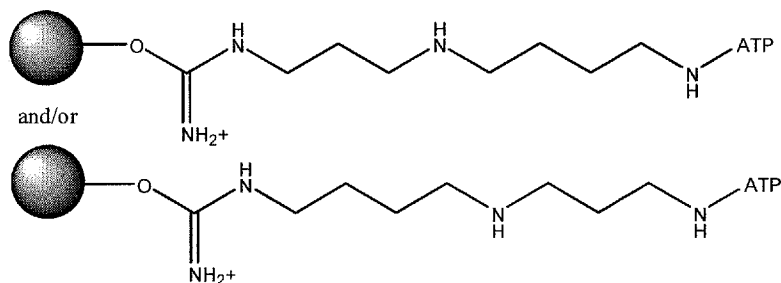
10 The resin was prepared by adding 260 mg 7-(2-amino-3-phenyl-propoxy)-heptylamine and 1.0 mL 1,4-dioxane to 4 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 5 hours at room temperature.

The affinity ligand was prepared by adding 480 mg ATP, 360  $\mu$ L 1-methyl  
15 imidazole and 840 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

## 20 EXAMPLE 77

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 77:



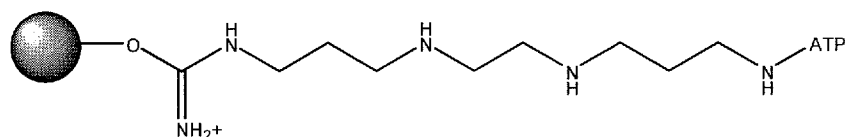
The resin was prepared by adding 105 mg spermidine and 0.5 mL 1,4-dioxane to 2.5 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

10 The affinity ligand was prepared by adding 240 mg ATP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 78

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 78:



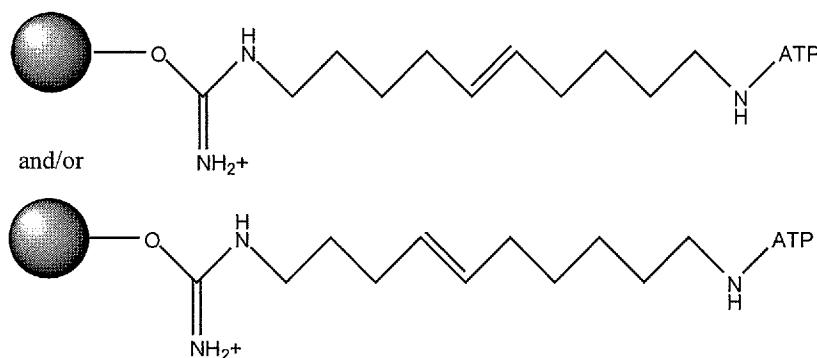
The resin was prepared by adding 127 mg bis(3-aminopropyl)-ethylene diamine and 0.5 mL 1,4-dioxane to 2.5 mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25 The affinity ligand was prepared by adding 240 mg ATP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

5           The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 79

10           The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 79:



15           1, 10 Diiodo-dec-5-ene is prepared as follows: 9-Borabicyclo[3.3.1]nonane (1 equiv.) is added to anhydrous THF at 0° C followed by followed by 1, 5, 9-decatriene (4 equiv.). The reaction is warmed to ambient temperature and stirred for 1.5 h. The resulting solution is cooled to -20° and sodium methoxide (2 equiv.) is added followed by iodine (2 equiv.). The reaction mixture is stirred at -20°C for 1.5 h, warmed to room temperature and stirred for an additional 1 h. The crude product is purified via silica gel chromatography. The appropriate fractions are pooled and concentrated in vacuo to afford 1, 10-Diiodo-dec-5-ene.

25           The 1, 10-Diiodo-dec-5-ene (1 equiv.) is dissolved in anhydrous acetone at ambient temperature followed by the addition of potassium phthalimide (4. equiv.). The reaction mixture is stirred until completion, washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The crude product is purified via silica gel chromatography to afford 1, 10-dipthalimido-dec-5-ene.

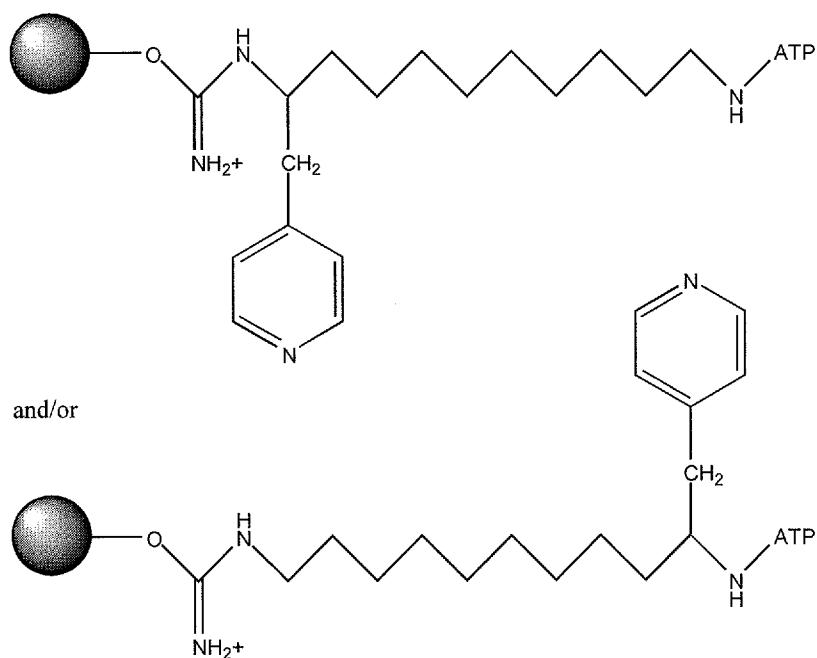
          The 1, 10-dipthalimido-dec-5-ene (1 equiv.) and hydrazine (2 equiv.) are stirred in ethanol at reflux. The reaction mixture is cooled to room temperature and the resulting

5 solid is filtered off. The filtrate is purified via silica gel chromatography to yield 1, 10 diamino-dec-5-ene.

200 mg of the linker arm and 1.5ml 1,4-dioxane are added to 3ml coupling buffer and combined with 500 mg cyanogen bromide-activated Sepharose. The reaction is allowed to proceed for about five hours. 480mg ATP, 360ml 1-methyl imidazole and 10 840mg EDC are added to 2.5ml water, allowed to react for one hour, and then combined the resin. The reaction is allowed to proceed overnight.

#### EXAMPLE 80

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 80:



Magnesium (1.2 equiv.) is stirred in anhydrous ether under a nitrogen atmosphere and a catalytic amount of iodine is added. The mixture is heated to reflux and 4-bromomethylpyridine (1 equiv.) is added. The reaction is stirred until formation of the Grignard reagent is complete and 9-phthalimidononanal (1.5 equiv.) is added. The reaction mixture is stirred until completion, quenched with ammonium chloride, extracted



5 with brine and concentrated in vacuo. The crude product is purified by silica gel chromatography to give 1-hydroxy-1-(4-pyridinyl)-10-phthalimidodecane.

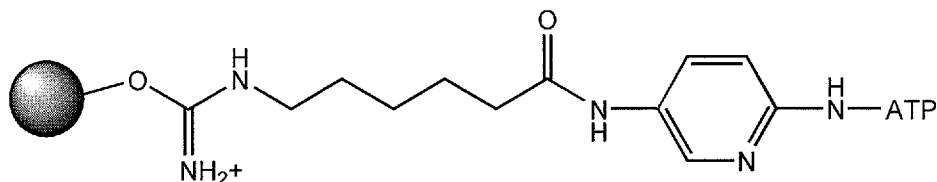
1-Hydroxy-1-(4-pyridinyl)-10-phthalimidodecane (1 equiv.) is dissolved in THF with stirring. Phthalimide (1.5 equiv.) and triphenylphosphine (2.1 equiv.) are added to the solution and the resulting mixture is cooled to 0° C. Diisopropyl azodicarboxylate  
10 (2.0 equiv.) is added dropwise to the above solution and the reaction is stirred until completion. The resulting solid is filtered off and the filtrate is concentrated in vacuo. The crude product is purified by silica gel chromatography to yield 1, 10-diphthalimido-1-pyridinyldecane.

1, 10-diphthalimido-1-pyridinyldecane (1 equiv.) and hydrazine (2 equiv.) are  
15 stirred in ethanol at reflux. The reaction mixture is cooled to room temperature and the resulting solid is filtered off. The filtrate is purified by silica gel chromatography to yield 1, 10-diamino-1-pyridinyldecane.

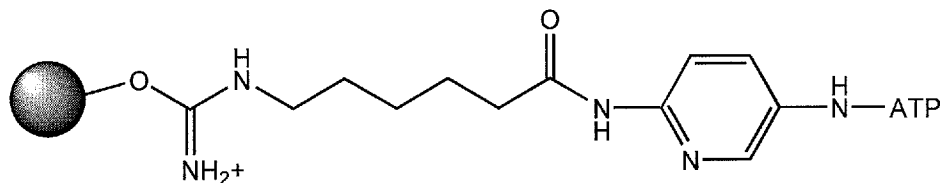
250mg of linker arm and 1.5ml 1, 4-dioxane is added to 3ml coupling buffer and combined with 500mg cyanogen bromide-activated Sepharose. The reaction is allowed  
20 to proceed for 5 hours. 480mg ATP, 360ml 1-methyl-imidazole and 840mg EDC are added to 2.5ml water, reacted for about one hour, and combined with the resin. The reaction is allowed to proceed overnight.

## 25 EXAMPLES 81-83

The following general structures represent the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 81-83:



5



CH-activated SEPHAROSE™ 4B (Sigma) is used for these examples.

#### EXAMPLE 81

10        50 mg of 2,5-diaminopyridine and 0.2 mL 1,4-dioxane are added to 0.8 mL coupling buffer and combined with 200 mg CH-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

      The affinity ligand is prepared by combining 100 mg ATP, 74 µL 1-methyl imidazole and 172 mg EDC to 1.5mL water, and reacting for approximately 1 hour at  
15    room temperature.

      The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 82

20        40 mg of 2,5-diaminopyridine, 10µL ethanolamine and 0.2 mL 1,4-dioxane are added to 0.8 mL coupling buffer and combined with 200 mg CH-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

      The affinity ligand is prepared by combining 100 mg ATP, 74 µL 1-methyl imidazole and 172 mg EDC to 1.5 mL water, and reacting for approximately 1 hour at  
25    room temperature.

#### EXAMPLE 83

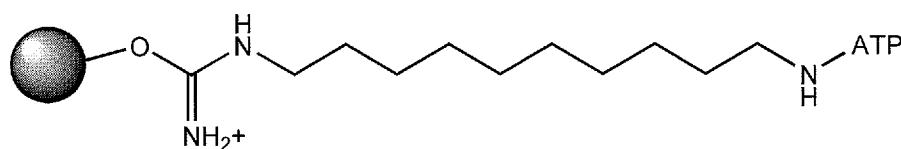
30        30 mg of 2,5-diaminopyridine, 20µL ethanolamine, and 0.2 mL 1,4-dioxane are added to 0.8 mL coupling buffer and combined with 200 mg CH-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

5 The affinity ligand is prepared by combining 100mg ATP, 74μL 1-methyl imidazole and 172 mg EDC to 1.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### 10 EXAMPLES 84-89

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 84-89:



Cyanogen bromide-activated SEPHAROSE™ 4B (Sigma) is used for these examples.

#### EXAMPLE 84

112.5 mg of 1,10-diaminodecane, 12.5 mg glycine, and 0.5 mL 1,4-dioxane are added to 2 mL coupling buffer and combined with 0.5 mg cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

25 The affinity ligand is prepared by combining 240 mg ATP, 180 μL 1-methyl imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 85

30 93.75 mg of 1,10-diaminodecane, 31.25 mg glycine, and 0.5 mL 1,4-dioxane are added to 2 mL coupling buffer and combined with 0.5 mg cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

5           The affinity ligand is prepared by combining 240 mg ATP, 180  $\mu$ L 1-methyl  
imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and the affinity ligand are combined and allowed to react for  
approximately 12-18 hours, or overnight, at room temperature.

10

#### EXAMPLE 86

          62.5 mg of 1,10-diaminodecane, 62.5 mg glycine, and 0.5 mL 1,4-dioxane are  
added to 2 mL coupling buffer and combined with 0.5 mg cyanogen bromide-activated  
SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room  
15   temperature.

          The affinity ligand is prepared by combining 240 mg ATP, 180  $\mu$ L 1-methyl  
imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and the affinity ligand are combined and allowed to react for  
20   approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 87

          112.5 mg of 1,10-diaminodecane, 12.5 mg tris(hydroxymethyl)aminomethane,  
and 0.5 mL 1,4-dioxane are added to 2 mL coupling buffer and combined with 0.5 mg  
25   cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for  
approximately 2 hours at room temperature.

          The affinity ligand is prepared by combining 240 mg ATP, 180  $\mu$ L 1-methyl  
imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at  
room temperature.

30           The resin and the affinity ligand are combined and allowed to react for  
approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 88

          93.75 mg of 1,10-diaminodecane, 31.25 mg tris(hydroxymethyl)aminomethane,  
35   and 0.5 mL 1,4-dioxane are added to 2 mL coupling buffer and combined with 0.5 mg

5 cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand is prepared by combining 240mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

10 The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 89

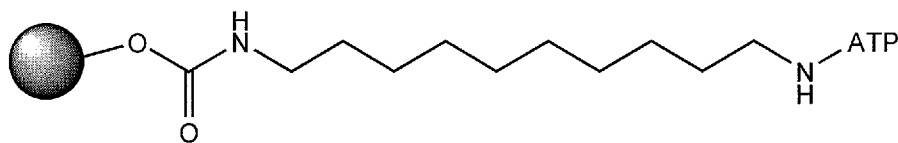
62.25mg of 1,10-diaminodecane, 62.25mg tris(hydroxymethyl)aminomethane,  
15 and 0.5mL 1,4-dioxane are added to mL coupling buffer and combined with 0.5 mg cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand is prepared by combining 240mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at  
20 room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLES 90-96

25 The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 90-96:



Trisacryl resin GF 2000 M (BioSeptra) or Ultrogel AcA 22 (BioSeptra) resin is  
30 used for these examples. The general procedure for these resins is the same as that described for the TOYOPEARL® and SEPHACRYL™ resins as described above.

5    EXAMPLE 90:

2.5 ml Trisacryl GF 2000 M resin was combined with 2.5 ml dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 250 mg of 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to  
10   proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1 M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at  
15   room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

EXAMPLE 91

20        2.5 ml Trisacryl GF 2000 M resin was combined with 2.5 ml dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 165 mg of 1,10-diaminodecane, 85 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL  
25   of a 1 M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at room temperature.

30        The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

EXAMPLE 92

2.5 ml Trisacryl GF 2000 M resin was combined with 2.5 ml dry acetone and 200  
35   mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1

5 hour at room temperature. 82 mg of 1,10-diaminodecane, 168  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5mL of a 1M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

10 The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

15

#### EXAMPLE 93

2.5 ml Ultrogel AcA 22 resin was combined with 2.5 ml dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 250 mg of 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 20 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1 M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl 25 imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### 30 EXAMPLE 94

2.5 ml Ultrogel AcA 22 resin was combined with 2.5 ml dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 165 mg of 1,10-diaminodecane, 85  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is 35 allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1M

5 ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10mL water, and reacting for approximately 1 hour at room temperature.

10 The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 95

2.5 ml Ultrogel AcA 22 resin was combined with 2.5 ml dry acetone and 200 mg  
15 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 82 mg of 1,10-diaminodecane, 168  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1M ethanolamine solution are then added to the resin and allowed to react at room  
20 temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand are combined and allowed to react for  
25 approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 96

2.5 ml Ultrogel AcA 22 resin was combined with 2.5 ml dry acetone and 200 mg  
1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour  
30 at room temperature. 25 mg of 1,10-diaminodecane, 225  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1 M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

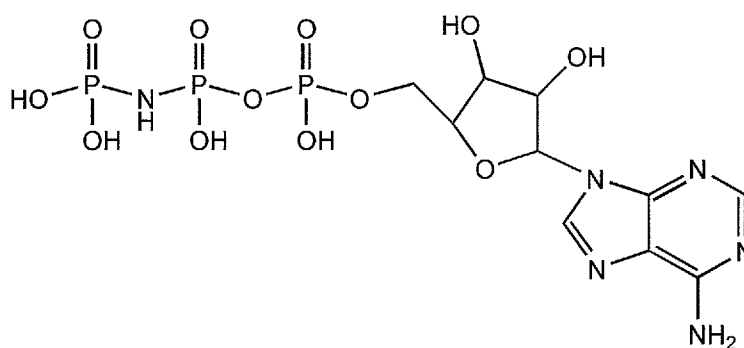


5           The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10mL water, and reacting for approximately 1 hour at room temperature.

          The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### 10       EXAMPLE 97

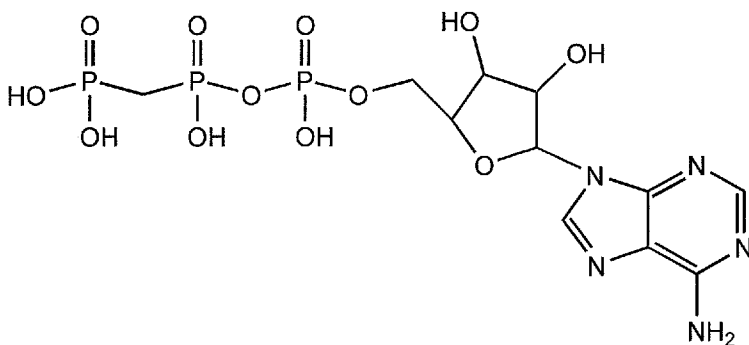
          The following structure represents an ATP analog out of which an alkyl linked nucleotide was made using the conditions described in Example 97:



15           13.5 mg of 1,10-diaminodecane, 13.5  $\mu$ L ethanolamine and 105  $\mu$ L 1,4-dioxane are added to 419  $\mu$ L coupling buffer and combined with 105 mg cyanogen-bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature. 29 mg adenylyl-imido-diphosphate (Calbiochem®, San Diego California), 22.5  $\mu$ L 1-methyl imidazole and 52.5 mg EDC are added to 625  $\mu$ L water, and the reaction  
20       is allowed to proceed for one hour. The resin is combined with the ligand, and the reaction is allowed to proceed overnight.

#### EXAMPLE 98

          The following structure represents an ATP analog out of which an alkyl linked  
25       nucleotide was made using the conditions described in Example 98:



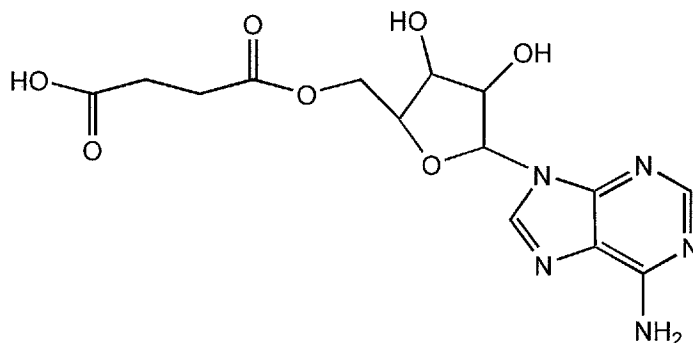
5

13.5 mg of 1,10-diaminodecane, 13.5  $\mu$ l ethanolamine and 105  $\mu$ l 1,4-dioxane are added to 419  $\mu$ l coupling buffer and combined with 105 mg cyanogen-bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature. 30 mg  $\beta$ , $\gamma$ -methylene-ATP (Calbiochem®, San Diego California), 22.5  $\mu$ l 1-methyl imidazole and 52.5 mg EDC are added to 625  $\mu$ l water, and the reaction is allowed to proceed for one hour. The resin is combined with the ligand, and the reaction is allowed to proceed overnight.

#### EXAMPLE 99

15 The following structure represents an ATP analog out of which an alkyl linked nucleotide was made using the conditions described in Example 99:

:



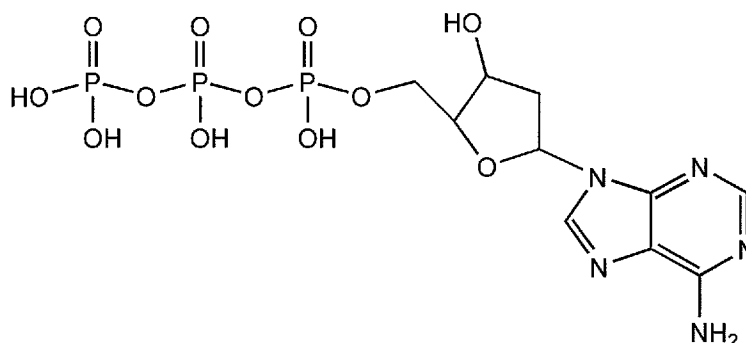
16 mg of 1,10-diaminodecane, 16  $\mu$ l ethanolamine and 125  $\mu$ l 1,4-dioxane are added to 500  $\mu$ l coupling buffer and combined with 125 mg cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature. 20 mg adenosine-5'-succinate (Sigma, St. Louis, MO), 22.5  $\mu$ l 1-methyl imidazole and 52.5 mg EDC are added to 625  $\mu$ l water, and the reaction is allowed to

5 proceed for one hour. The resin is combined with the ligand, and the reaction is allowed to proceed overnight.

#### EXAMPLE 100

The following structure represents an ATP analog out of which an alkyl linked  
10 nucleotide was made using the conditions described in Example 100:

:



16 mg of 1,10-diaminodecane, 16  $\mu$ l ethanolamine and 125  $\mu$ l 1,4-dioxane are added to 500  $\mu$ l coupling buffer and combined with 125 mg cyanogen-bromide-activated  
15 SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature. 29 mg 2-deoxy-ATP (Sigma, St. Louis, MO), 22.5  $\mu$ l 1-methyl imidazole and 52.5 mg EDC are added to 625  $\mu$ l water, and the reaction is allowed to proceed for one hour. The resin is combined with the ligand, and the reaction is allowed to proceed overnight.

20

#### EXAMPLES 101-105

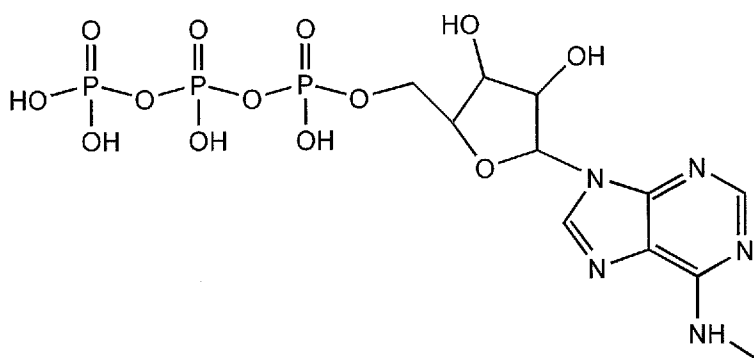
The alkyl-linked nucleotide ~~compositions~~ non-homogeneous solid supports of Examples 101-105 are made in three steps: the adenosine derivative is prepared; phosphorylated, and then attached to the resin-linker arm combination. For each of  
25 examples 99-103, the adenosine derivatives are phosphorylated as follows. The adenosine derivatives are dissolved in trimethyl phosphate (5-12 mL) and cooled to about 0°C. After stirring for 10 minutes, phosphorous oxychloride (1.8 equivalents) is added dropwise. The reaction mixture is stirred at 0°C for 6 hours. Tributylamine (5.4 equivalents) is added followed immediately by 0.5 M tributylammonium pyrophosphate

5 (4 equivalents) and stirring is continued for about 15 additional minutes. The reaction is quenched by the addition of 0.2 M triethylammonium bicarbonate (40-50 mL). The reaction mixture is then stirred at room temperature for 15 hours, and then lyophilized. The crude preparation is then purified on a Sephadex™ DEAE-25-ion-exchange resin, and eluted with water following a gradient of TEAB buffer (0.05 M to 0.5 M). The  
10 appropriate fractions are then combined.

#### EXAMPLE 101

The following structure represents an ATP analog synthesized using the conditions described in Example 101:

15

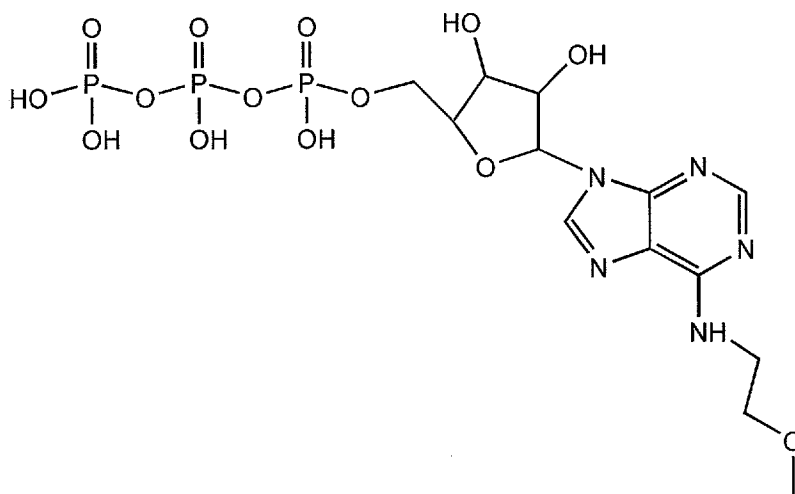


1.016 g 6-chloropurine riboside and 8.82 mL methylamine (33% in ethyl alcohol) are combined and heated to 90°C in a sealed reaction vessel for about 22 hours. The  
20 reaction vessel is cooled in an ice bath for about 30 minutes to allow formation of a solid precipitate. The precipitate is filtered and washed with ice-cold ethyl alcohol (3 × 25 mL) and dried to 0.66 g of *N*<sup>6</sup>-methyladenosine in 66% yield.

16 mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated  
25 Sepharose™. The reaction is allowed to proceed for about 2 hours. 202 mg ligand, 90 µl 1-methyl imidazole and 210 mg EDC are added to 1 ml water and allowed to react to about one hour. The ligand is then added to the resin and the reaction is allowed to proceed overnight.

5    EXAMPLE 102

The following structure represents an ATP analog synthesized using the conditions described in Example 102:



10

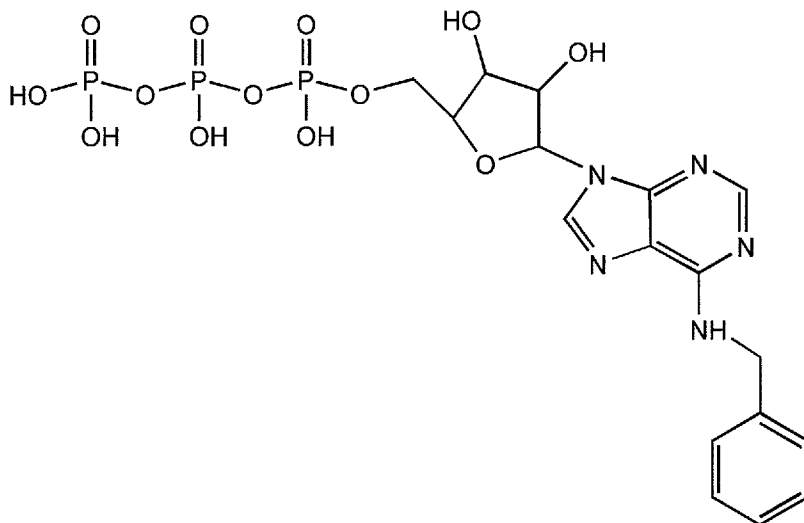
547 mg 6-chloropurine riboside, 0.882 mL 2-methoxyethylamine, and 5.0 mL ethyl alcohol are combined and heated to 90°C in a sealed reaction vessel for about 22 hours. The ligand is then purified by silica gel chromatography using 8:1 methylene chloride:methanol to yield *N*<sup>6</sup>-(2-methoxyethyl)-adenosine (0.61g, 98% yield).

15        16mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated Sepharose™. The reaction is allowed to proceed for about 2 hours. 154 mg ligand, 90 µl 1-methyl imidazole and 210 mg EDC are added to 1 ml water and allowed to react to about one hour. The ligand is then added to the resin and the reaction is allowed to  
20    proceed overnight.

EXAMPLE 103

The following structure represents an ATP analog synthesized using the conditions described in Example 103:

25



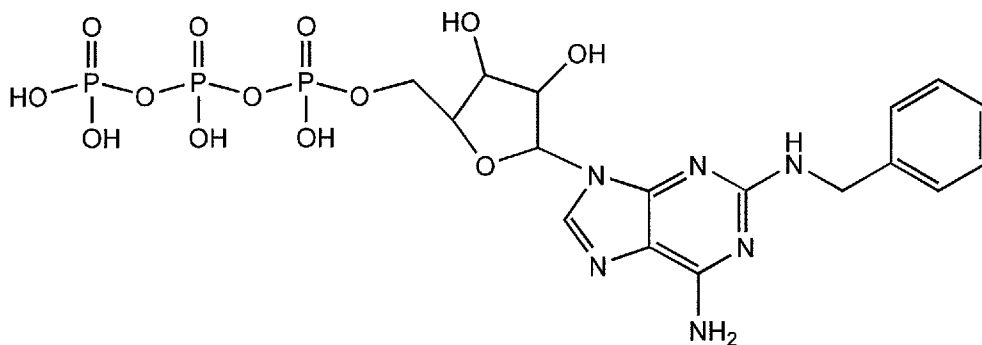
5

473 mg 6-chloropurine riboside, 0.54 mL 2-benzylamine, 0.69 ml triethylamine, and 5.0 mL of ethyl alcohol are combined and heated to 90°C in a sealed reaction vessel for about 18 hours. The reaction vessel is cooled in an ice bath for about 30 minutes to allow formation of a solid precipitate. The precipitate is filtered and washed with ice-cold ethyl alcohol (3 × 25 mL) and dried to yield 0.51 g of *N*<sup>6</sup>-benzyladenosine (86% yield).

16 mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated Sepharose™. The reaction is allowed to proceed for about 2 hours. 160.8 mg ligand, 90 µl 1-methyl imidazole and 210 mg EDC are added to 1 ml water and allowed to react to about one hour. The ligand is then added to the resin and the reaction is allowed to proceed overnight.

## 20 EXAMPLE 104

The following structure represents an ATP analog synthesized using the conditions described in Example 104:



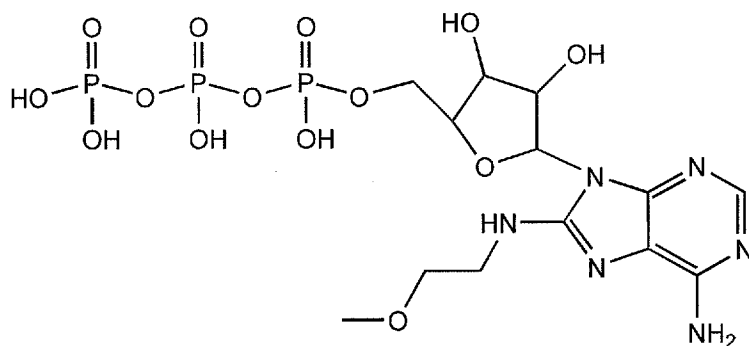
5

400 mg 2-chloroadenosine hemihydrate, 0.42 mL 2-benzylamine, 0.54 ml triethylamine, and 5.0 mL of ethyl alcohol are combined and heated to 90°C in a sealed reaction vessel for about 96 hours. The ligand is then purified by silica gel chromatography using 20:1; methylene chloride:methanol to yield 0.11 g of *N*<sup>2</sup>-benzyladenosine (22%).

16 mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated Sepharose™. The reaction is allowed to proceed for about 2 hours. 120 mg ligand, 67.5 µl 1-methyl imidazole and 157.5 mg EDC are added to 1 ml water and allowed to react to about one hour. The ligand is then added to the resin and the reaction is allowed to proceed overnight.

#### EXAMPLE 105

20 The following structure represents an ATP analog synthesized using the conditions described in Example 105:



5           507 mg 8-bromoadenosine, 0.631 mL 2-methoxyethylamine, and 5.0 mL of ethyl alcohol are combined and heated to 90°C in a sealed reaction vessel for about 168 hours. The ligand is then purified by silica gel chromatography using 4:1, methylene chloride:methanol to yield 0.52 g of *N*<sup>8</sup>-(2-methoxyethyl)-adenosine (99% yield) as a clear, viscous oil.

10           16 mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated Sepharose™. The reaction is allowed to proceed for about 2 hours. 157.2 mg ligand, 90 µl 1-methyl imidazole and 210 mg EDC are added to 1 ml water and allowed to react to about one hour. The ligand is then added to the resin and the reaction is allowed to  
15   proceed overnight.

          While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that  
20   various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

          Various publications, patent applications and patents are cited herein, the disclosures of which are incorporated by reference in their entireties.

25



## ALKYL-LINKED NUCLEOTIDE COMPOSITIONS

## ABSTRACT OF THE DISCLOSURE

10        Alkyl-linked nucleotide ~~compositions~~ non-homogeneous solid supports and  
nucleotide affinity media comprising an alkyl-linked nucleotide are provided. The linker  
is generally a hydrophobic linker that can be a 3, 4, 5, 6, 7, 8, 9, 10, or a longer carbon  
chain. Also included in the invention are methods for synthesis of an alkyl-linked  
15    nucleotide, nucleotide affinity media and methods of use thereof for affinity  
chromatography and screening methods.

## ALKYL-LINKED NUCLEOTIDE COMPOSITIONS

### 5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Applications 60/453,697, filed January 22, 2003, and 60/532,134, filed December 23, 2003, each of which are hereby incorporated in its entirety by reference herein.

### 10 FIELD OF THE INVENTION

The present invention relates to nucleotide affinity media and methods for their use.

### BACKGROUND OF THE INVENTION

15 Previous methods of preparing ligands, such as nucleotides, for use in affinity chromatography have typically coupled the nucleotide to a solid matrix through the N6 amino group on the purine ring, or via a hydroxyl group of the ribose moiety. However, these ligands are not always effective ligands for affinity chromatography, usually because of steric hindrance or the orientation of the ligand on the solid matrix. The  
20 studies of the molecular structures of some nucleotide binding proteins, such as kinases, support these findings.

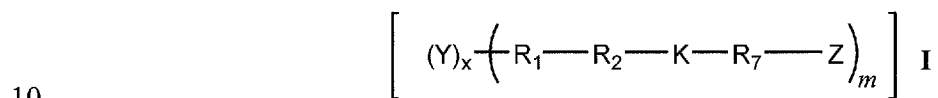
Recently, in an alternative method, the nucleotide, adenosine triphosphate (ATP), was coupled to an affinity resin indirectly through the gamma-phosphate group of ATP via an aminophenyl moiety. Linking ATP to a resin via an aminophenyl group attached  
25 to the gamma-phosphate of ATP has advantages over earlier nucleotide affinity media.

However, a need still exists to develop a still more efficient method for synthesis of nucleotide affinity media that are suitable for use in affinity chromatography and screening methods.

## SUMMARY OF THE INVENTION

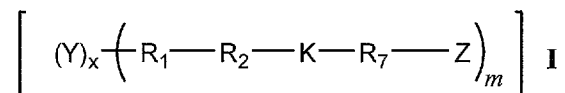
The present invention is directed to alkyl-linked nucleotide non-homogeneous solid supports, which include alkyl-linked nucleotide affinity media.

In one embodiment, an alkyl-linked nucleotide non-homogeneous solid support has the general formula:



such that: Y is a solid support, a tag, or a protective group; x is either 0 or 1; R<sub>1</sub> is a covalent bond between Y and R<sub>2</sub>, or R<sub>1</sub> is an acyl group, a substituted or a non-substituted group and the substituted or non-substituted group is selected from an alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, aryl, or a heteroaryl group, or a combination of these groups; R<sub>2</sub> is a substituted or a non-substituted group, and the substituted or non-substituted group is selected from an alkyl, cycloalkyl, heteroalkyl, heterocycloalkyl, or a heteroaryl group, or a combination of these groups; K is a heteroatom; R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate and n is at least one or R<sub>7</sub> is a phosphate group mimic, Z is a nucleoside or nucleoside derivative; and m is at least one.

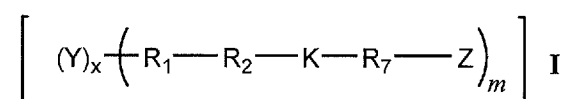
Also included in the invention is a method to synthesize an alkyl-linked nucleotide non-homogeneous solid support comprising an alkyl-linked nucleotide affinity medium, said alkyl-linked nucleotide affinity medium having a general formula:



comprising the general steps of (a) coupling at least one linker to a solid support or tag in a suitable coupling buffer, wherein the linker is R<sub>2</sub>, or a combination of R<sub>1</sub> and R<sub>2</sub>; (b) end-capping reactive sites remaining on the solid support or tag after the coupling step; and (c) reacting a terminal phosphate or thiophosphate group of a nucleotide with the linker coupled to the solid support or tag, wherein Y is a solid support or a tag; x = 1; R<sub>1</sub> is a covalent bond between Y and R<sub>2</sub>, or R<sub>1</sub> is an acyl group, a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof; R<sub>2</sub> is a substituted or a non-substituted alkyl

5 group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof; K is a heteroatom; R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate and n is at least one or R<sub>7</sub> is a phosphate group mimic, Z is a nucleoside or nucleoside derivative; and m is at least  
10 one.

Also included in the invention is a method for screening compounds. For example, the method comprises the steps of (a) contacting a proteome with a nucleotide affinity medium comprising a general formula:



15 wherein Y is a solid support or a tag; x = 1; R<sub>1</sub> is a covalent bond between Y and R<sub>2</sub>, or R<sub>1</sub> is an acyl group, a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof;  
20 R<sub>2</sub> is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof; K is a heteroatom; R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate and n is at least one or R<sub>7</sub> is a phosphate group mimic, Z is a nucleoside or  
25 nucleoside derivative; and m is at least one; (b) washing the nucleotide affinity medium with a buffer, whereby non-specifically bound components of the proteome are eluted from the nucleotide affinity medium and specific components of the proteome remain bound to the nucleotide affinity medium; (c) contacting the nucleotide affinity medium bound to specific components of the proteome with at least one test compound; (d)  
30 eluting from the nucleotide affinity medium components of the proteome that are specifically displaced by the test compound; and (e) identifying the components of the proteome that are specifically displaced by the test compound from the nucleotide affinity medium.

5           The alkyl-linked nucleotide non-homogeneous solid supports and alkyl-linked  
nucleotide affinity media as described herein are particularly useful, for example, as  
affinity ligands in affinity chromatography methods, for the screening of proteomes or  
combinatorial libraries, and for the purification of compounds such as, for example,  
10       proteins. Furthermore, the invention includes a more efficient method for the synthesis  
of such alkyl-linked nucleotides and nucleotide affinity media than has been previously  
accomplished.

#### BRIEF DESCRIPTION OF THE DRAWINGS

15       Fig. 1 is a schematic of the synthesis of a compound Intermediate IA using  
cyanogen bromide-activated beaded agarose and a linker.

Fig. 2 is a schematic of the synthesis of a compound Intermediate IB using 1,1'-  
carbonyldiimidazole (CDI)-activated beaded agarose and a linker.

Fig. 3 is a schematic of the synthesis of a compound Intermediate II.

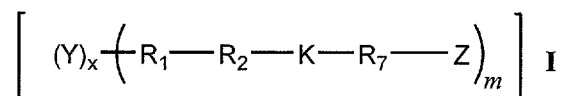
20       Fig. 4 is a schematic of the synthesis of a  $\gamma$ -phosphate-linked ATP using  
Intermediates IA and II as reaction components.

Fig. 5 is a schematic of the synthesis of a  $\gamma$ -phosphate-linked ATP using  
Intermediates IB and II as reaction components.

#### DETAILED DESCRIPTION OF THE INVENTION

25       The features and other details of the invention, either as steps of the invention or  
as combinations of parts of the invention, will now be more particularly described and  
pointed out in the claims. It will be understood that the particular embodiments of the  
invention are shown by way of illustration and not as limitations of the invention. The  
principle features of this invention can be employed in various embodiments without  
30       departing from the scope of the invention.

The present invention relates to an alkyl-linked nucleotide non-homogeneous  
solid support comprising the general formula:



5 wherein Y is a solid support, a tag, or a protective group;  $x = 0$  or  $1$ ;  $R_1$  is a covalent bond  
between Y and  $R_2$ , or  $R_1$  is an acyl group, a substituted or a non-substituted alkyl group, a  
substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted  
heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted  
or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a  
10 combination thereof;  $R_2$  is a substituted or a non-substituted alkyl group, a substituted or  
a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a  
substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted  
heteroaryl group, or a combination thereof; K is a heteroatom;  $R_7$  is  $(P)_n$  where P is a  
phosphate or thiophosphate and n is at least one or  $R_7$  is a phosphate group mimic, Z is a  
15 nucleoside or nucleoside derivative; and m is at least one.

Such substituted and non-substituted alkyl, cycloalkyl, heteroalkyl,  
heterocycloalkyl, aryl, heteroaryl groups, and combinations of same, can be linear or  
branched chains, as will be understood by one of skill in the art.

A "heteroaryl," as that term is used herein, is an aryl group that includes at least  
20 one aromatic ring structure in which one or more of the atoms of at least one of the  
aromatic rings is an element other than carbon, for example, sulfur, nitrogen or oxygen.  
Examples of heteroaromatic compounds include pyridine, pyrimidine, oxazole, quinoline,  
thiophene and furan.

A heteroatom (K) is preferably a nitrogen atom (N), an oxygen atom (O), or a  
25 sulfur atom (S). Preferably, the heteroatom (K) is a nitrogen atom.

An alkyl-linked nucleotide is also referred to herein as a ligand or an affinity  
ligand.

An alkyl-linked nucleotide bound to a solid support or a tag, such that the solid  
support or tag is suitable for the separation of the alkyl-linked nucleotide, and optionally,  
30 compounds (such as proteins, for example) bound to the alkyl-linked nucleotide, from  
unbound compounds, is also referred to herein as a "nucleotide affinity medium or  
media", or as an "alkyl-linked nucleotide affinity medium or media".

A solid support (Y) can be any suitable support, such as a resin, or a particulate  
material, such as a bead, or a particle. Alternatively, a solid support can be a continuous  
35 solid surface, such as a plate, chip, well, channel, column or a tube. The material of a

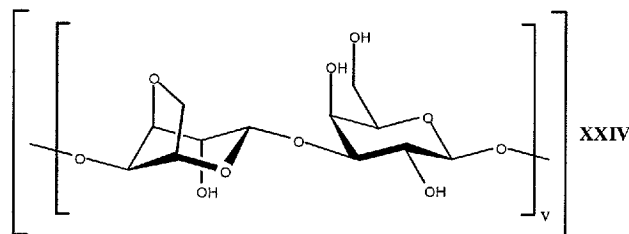
5 solid support will be of any suitable substance, compound or polymer, as will be appreciated by one of skill in the art. Examples include, without limitation, acrylamide, agarose, methacrylate polymers, methacrylate copolymers, cellulose, nylon, silica, glass, ceramic, a magnetized particle or surface, nitrocellulose, polystyrene, thermoresponsive polymers (see, for example, Lee, et al. (1996) *Journal of Applied Polymer Science* 62: 301-311; Yoshida, et.al. (1996) *Macromolecules* 29:8987-89; and Osada and Khokhlov, eds. (2001) *Polymer Gels and Networks* (Marcel Dekker, New York); each of which is  
10 herein incorporated by reference in its entirety), and derivatives thereof.

In one embodiment, the solid support (Y) is a SEPHACRYL™ resin. SEPHACRYL™ is an acrylamide derivative, produced by polymerizing allyl dextran  
15 with the cross-linking monomer N,N'-methylene-bisacrylamide.

In another embodiment, the solid support (Y) is a TOYOPEARL® resin. TOYOPEARL® is a methacrylate derivative, produced by the co-polymerization of glycidyl methacrylate, pentaerythritol dimethylmethacrylate and polyethylene glycol.

In one embodiment, the solid support (Y) is a beaded agarose. An example of a  
20 suitable beaded agarose is SEPHAROSE™ beaded agarose. Beaded agarose, such as SEPHAROSE™ beaded agarose, can be a cross-linked preparation, such as will be appreciated by one of skill in the art. Cross-linked preparations are generally recognized to have good chemical and physical stability properties. The choice of a suitable solid support will be apparent to one of skill in the art from the known characteristics of a solid  
25 support and the method of use of that solid support.

Agarose is a linear polymer with a basic structure as follows:



wherein v is at least one. Variations of this basic structure of agarose will be recognized by one of skill in the art. The preparation and use of solid supports, such as agarose, are  
30 well known in the art (see, for example, Cuatrecasas and Anfinsen, "Affinity Chromatography" in Ann. Rev. Biochem. Snell et al., eds. (CA: Annual Reviews Inc.),

5 40: 259-278 (1971), the teachings of which are incorporated herein by reference in their entirety).

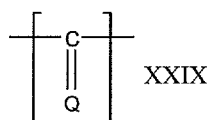
As used herein, a tag is an agent that provides for the specific detection or capture of the alkyl-linked nucleotide. For example, and without limitation, a suitable tag is biotin, avidin, streptavidin, a hapten, a fluorophore or a chromophore. Detection or  
10 capture of the alkyl-linked nucleotide employs techniques that are standard in the art. For example, a biotin-tagged alkyl-linked nucleotide can be captured using avidin, streptavidin or related avidin derivatives. The biotin-avidin interaction is highly specific. An avidin-conjugated agent used to detect or capture a biotin-tagged alkyl-linked nucleotide can be soluble (for example, an antibody), or a particulate material (for  
15 example, beaded agarose or a magnetized particle), or a continuous surface (for example, a plate or well coated with avidin). Detection and capture of a hapten-tagged alkyl-linked nucleotide can be achieved, for example, using hapten-specific antibodies. Visual detection methods for fluorophore or chromophore-tagged alkyl-linked nucleotides are readily understood by one of skill in the art.

20 Protective groups are well-known and standard in the art. The selection of a protective group will be dependent upon the properties of the reactive group, the conditions in which the compound is to be used and the function that is desired. These are readily understood by those of skill in the art (see, generally, "Protective Groups in Organic Synthesis" Greene and Wuts, eds. (NY: John Wiley & Sons, Inc.) 3rd edition  
25 (1999), the teachings of which are incorporated herein by reference in their entirety). Protective groups are used to selectively protect reactive groups such as hydroxyl, amino, carboxyl, carbonyl, sulfhydryl, and phosphate groups.

$R_1$  can be a covalent bond, or when  $R_1$  is not a covalent bond,  $R_1$  is also referred to herein as a linker or linker arm.  $R_2$  is also referred to herein as a linker or a linker arm.  
30 A linker can be selected from any suitable alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, substituted, non-substituted, linear or branched group, or a combination of same. Generally, the linker is a hydrophobic linker. For example, the linker can be a 3, 4, 5, 6, 7, 8, 9, 10, or longer carbon chain. Furthermore, a linker used in the invention can be highly hydrophobic or moderately hydrophobic, as will be  
35 understood by one of skill in the art. Alternatively, a hydrophilic linker can be used.

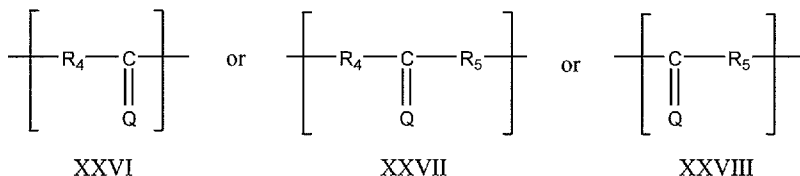


5 In one embodiment, R<sub>1</sub> comprises:



wherein Q = O or NH<sub>2</sub><sup>+</sup>.

In another embodiment, R<sub>1</sub> comprises:



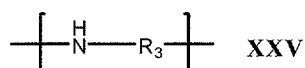
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wherein Q = O or NH<sub>2</sub><sup>+</sup>; R<sub>4</sub> is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof; and R<sub>5</sub> is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof.

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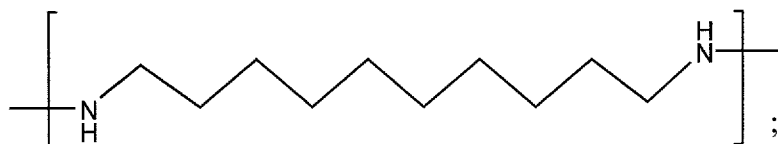
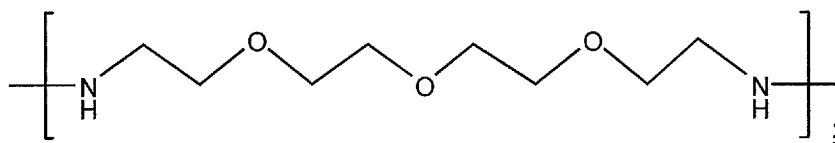
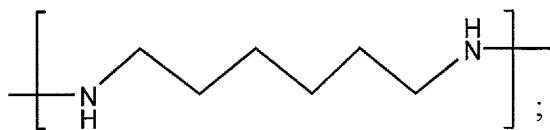
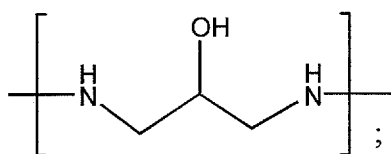
In one embodiment, R<sub>2</sub> comprises the general formula:



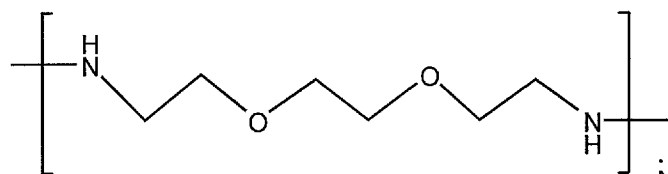
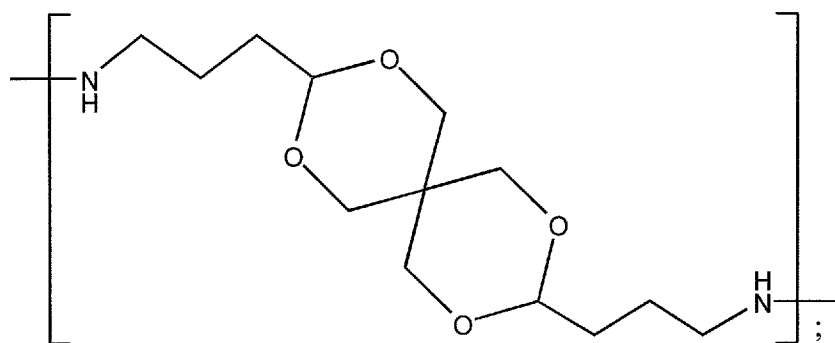
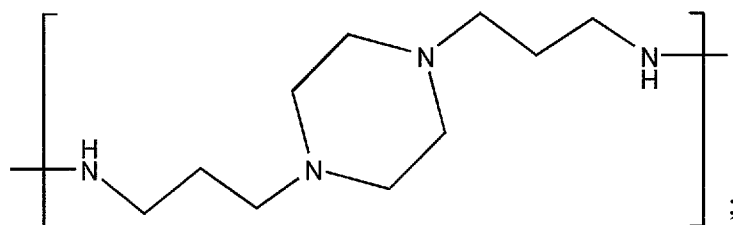
wherein R<sub>3</sub> is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

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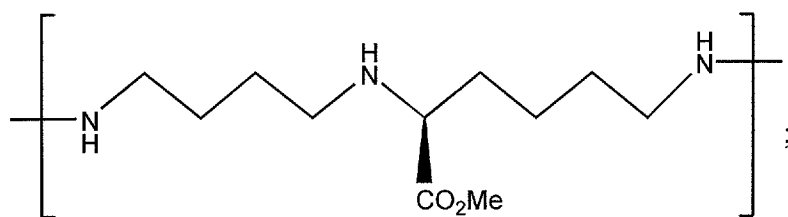
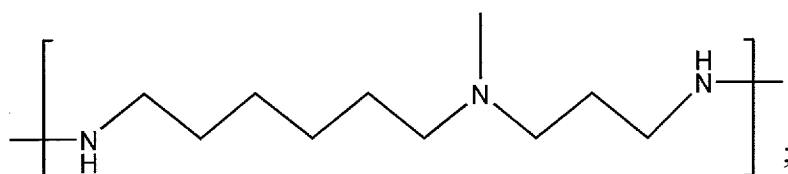
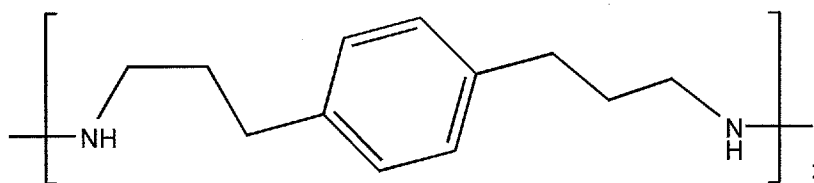
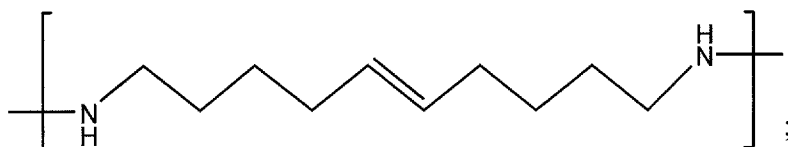
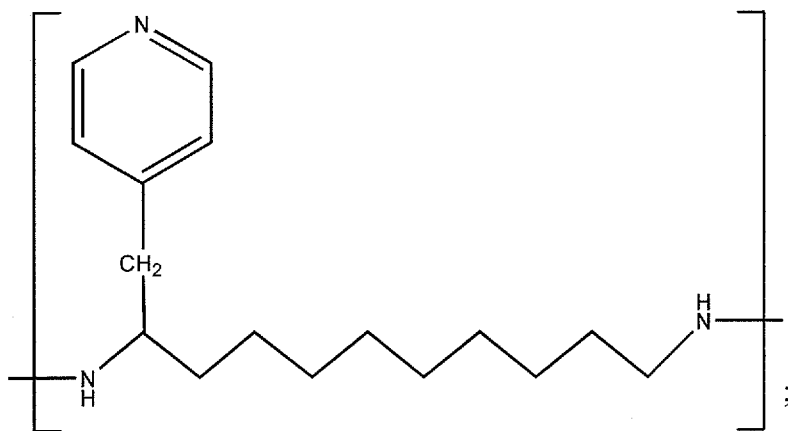
5            Examples of suitable linkers include, without limitation:



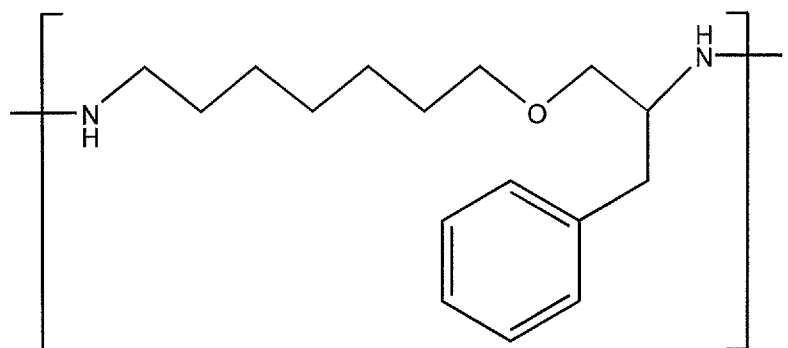
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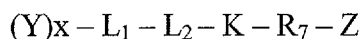
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5           Furthermore, a linker, such as described above, can be attached (for example, via a condensation reaction) to another linker to form a larger and/or a longer linker. For example, a linker can be formed by the tandem synthesis of linkers in a linear configuration. This can be represented, for example, as:

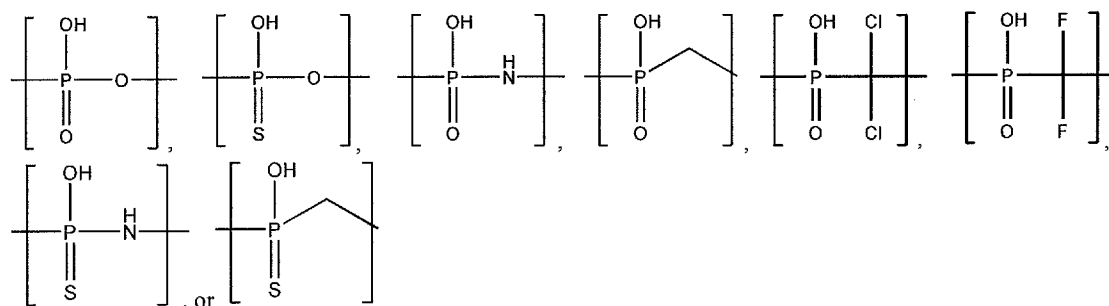


10       where Y is a solid support, a tag or a protective group, x is 0 or 1; L<sub>1</sub> and L<sub>2</sub> are linkers, such as provided in the above examples, and they can be the same or different linkers; K is a heteroatom; R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate and n is at least one or R<sub>7</sub> is a phosphate group mimic, and Z is a nucleoside or nucleoside derivative. As shown in the above examples, two linkers can be synthesized in tandem, however, it will be  
15       understood that two, three, or more linkers, can be synthesized in tandem. Alternatively, a branched configuration of linkers can be synthesized. Again, the linkers can be the same or different. Different linkers can be chosen according to their different hydrophobic or hydrophilic properties, as will be understood by one of skill in the art.

          Additionally or alternatively, more than one alkyl-linked nucleotide can be bound  
20       to Y, for example when Y is a solid support, by more than one type of linker. Again, different linkers can be chosen according to their different hydrophobic or hydrophilic properties, as will apparent to one of skill in the art. Thus, for example, when Y is a solid support, more than one nucleotide or nucleotide derivative (which may be the same nucleotide or different nucleotides, for example, only ATP, or a mixture of AMP and  
25       ADP, etc.) can be bound to the solid support by linkers that have similar or different hydrophobic properties (or hydrophilic properties). Examples of suitable synthesis methods for these affinity media are provided in the exemplification.

          In some embodiments of the invention, R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate group and n ≥ 1. Examples of suitable phosphate and thiophosphate  
30       groups that may be used include, without limitation:

5

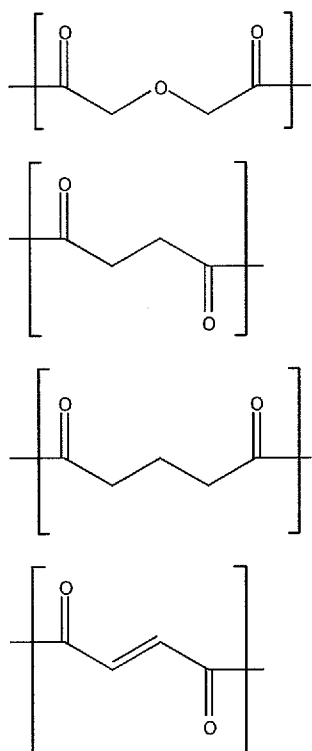


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As will be understood by one of skill in the art, phosphate and thiophosphate groups can also be present in an ionized variant or salt form. In some embodiments of the invention  $n$  is  $\geq 1$ ,  $\geq 2$ ,  $\geq 3$ , or  $\geq 4$ . For example,  $n$  can be 1, 2, 3, or 4. When  $n > 1$ , any combination of phosphate or thiophosphate groups may be used.

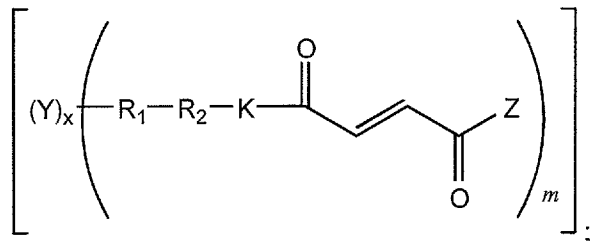
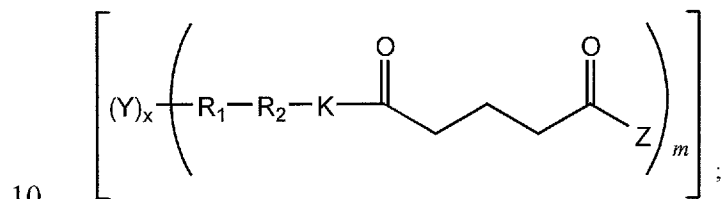
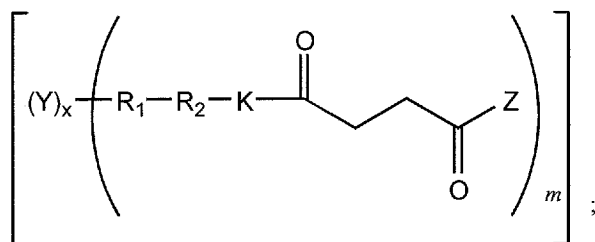
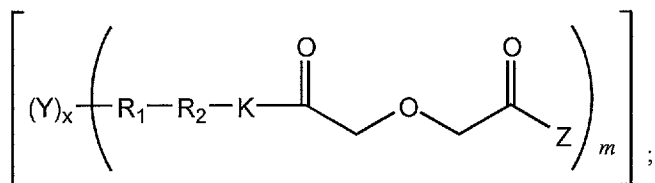
In other embodiments of the invention,  $R_7$  is a phosphate group mimic. For example, in some embodiments,  $R_7$  is a carboxylic acid that contains 4-8 carbons in the main chain and optionally contains a heteroatom. Examples of suitable carboxylic acids include, without limitation:

15

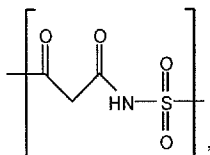
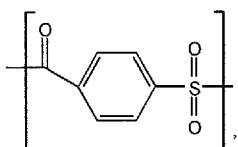


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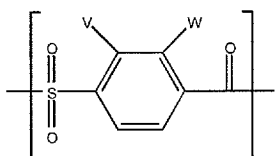
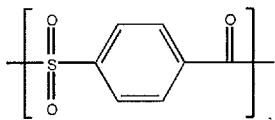
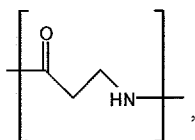
5 Accordingly, in some embodiments the alkyl linked nucleotide compositions have a general formula selected from:



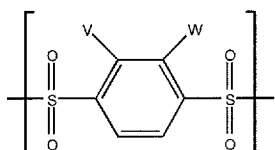
15 Other phosphate group mimics that may be used according to the invention include, without limitation, the following:



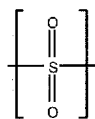
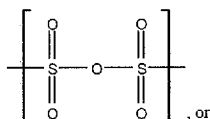
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where V and W can be H or a heteroatom such as NH<sub>2</sub> or OH,



where V and W can be H or a heteroatom such as NH<sub>2</sub> or OH,



10

A nucleoside and a nucleotide, as referred to herein, can be naturally-occurring or non-naturally-occurring. Furthermore, the nucleoside or nucleotide can be biologically or synthetically-derived using techniques that are standard to one of skill in the art. Suitable nucleosides include, without limitation, adenosine (A), guanosine (G), cytidine (C), thymidine (T) and uridine (U), and derivatives and analogs thereof.

Nucleotides are nucleosides with at least one phosphate group (or thiophosphate group), for example, a monophosphate, diphosphate or triphosphate group. The nucleotide can have phosphate or thiophosphate groups, or a combination of both. The number of phosphate or thiophosphate groups is at least one, and can be one, two, three or more in number. Such nucleotides are often referred to in abbreviation, for example, AMP, ADP, ATP, GMP, GDP, GTP, etc., as is understood by one of skill in the art.

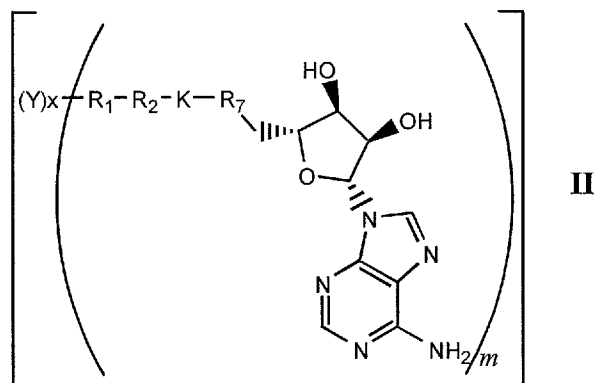
5           In one embodiment, the nucleotide is a monophosphate, diphosphate, or triphosphate of adenosine, guanosine, cytidine, thymidine, or uridine.

          Nucleoside and nucleotide derivatives and analogs are also encompassed by the invention. The isolation or synthesis of nucleoside derivatives and analogs are accomplished using techniques that are standard in the art, see for example, Guranowski  
10 et al. (1981) *Biochemistry* 20:110-15; Yaginuma *et al.* (1981) *J. Antibiot.* 23:359-66; Robins *et al.* (1983) *J. Am. Chem. Soc.* 105:4059-65; Borchardt *et al.* (1984) *J. Biol. Chem.* 259:5353-58; De Clercq *et al.* (1987) *Biochem. Pharmacol.* 36:2567-75; Seela *et al.* (1991) *Helv. Chim. Acta* 74:1048; Franchetti *et al.*, (1994) *J. Med. Chem.* 37: 3534-3541; Van Calenberg *et al.* (1994) *Helv. Chim. Acta.* 77:631-44; Picher *et al.* (1996)  
15 *Biochem. Pharmacol.* 51:1453- 601; Rosse *et al.* (1997) *Helv. Chim. Acta.* 80:653; Cowart *et al.* (1999) *J. Org. Chem.* 64:2240-49. Fischer *et al.*, (1999) *J. Med. Chem.* 42:3636-3646; van Tilburg *et al.* (1999) *J. Med. Chem.* 43:1393- 400; Halbfinger *et al.*, (1999) *J. Med. Chem.* 42:5325-5337; Ingall *et al.* (1999) *J. Med. Chem.* 42:213-20; Gendron *et al.*, (2000) *J. Med. Chem.* 43:2239-2247; Loog *et al.* (2000) *FEBS Letters*  
20 480:244; Bressi *et al.* (2001) *J. Med. Chem.* 44:2080-93; Herforth *et al.* (2002) *J. Comb. Chem.* 4:302-14; Hernandez *et al.* (2002) *J. Med. Chem.* 45:4254-63; Parang *et al.* (2002) *Pharmacology and Therapeutics* 93:145; Xu *et al.* (2002) *J. Med. Chem.* 45:5694-709; Hocek and Dvorakova (2003) *J. Org. Chem.* 68:5773-6; Koroniak *et al.* (2003) *Pharmacology & Therapeutics* 93:145; and Kourafalos *et al.* (2003) *J. Org. Chem.*  
25 68:6466-69; the teachings of all of which are incorporated herein by reference in their entirety.

          In one embodiment, the invention includes an alkyl-linked nucleotide non-homogeneous solid support comprising an alkyl-linked adenosine, said alkyl-linked nucleotide non-homogeneous solid support comprising the general structure:



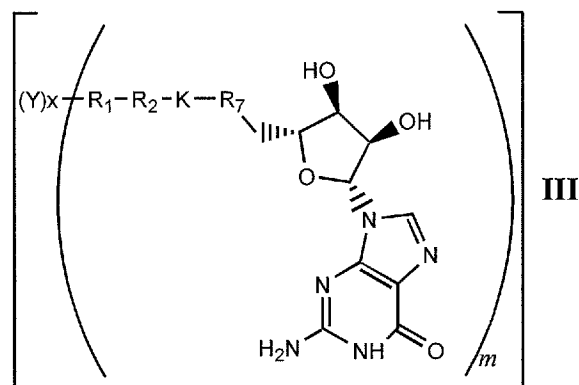
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or an ionized variant or a salt thereof.

In another embodiment is an alkyl-linked nucleotide non-homogeneous solid support comprising an alkyl-linked guanosine, said alkyl-linked nucleotide non-homogeneous solid support comprising the general structure:

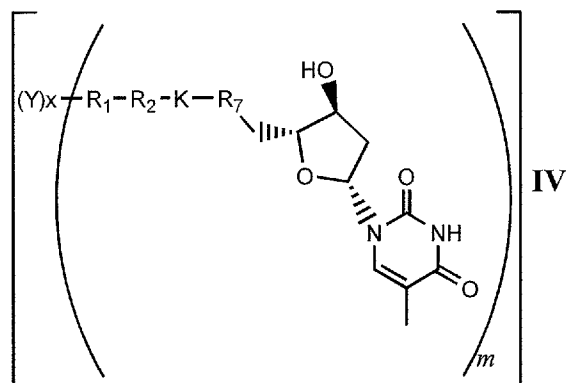
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or an ionized variant or a salt thereof.

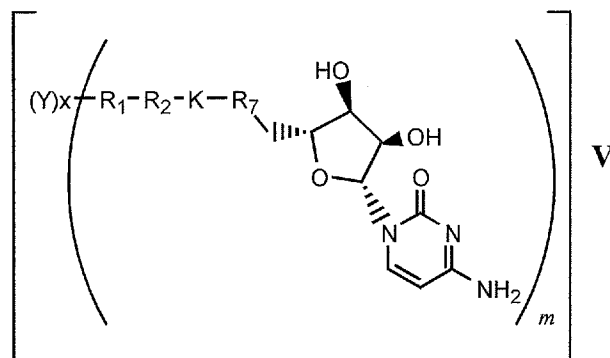
In another embodiment is an alkyl-linked nucleotide non-homogeneous solid support comprising an alkyl-linked thymidine, said alkyl-linked nucleotide non-homogeneous solid support comprising the general structure:

15



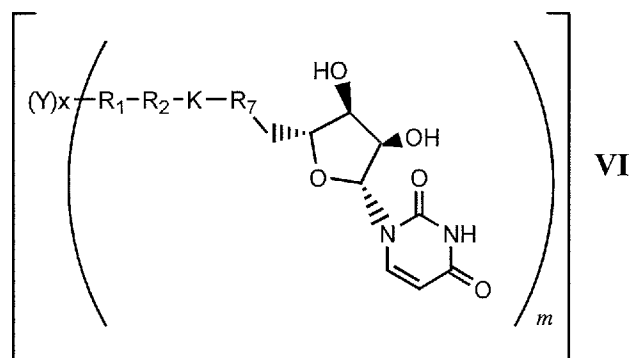
5 or an ionized variant or a salt thereof.

In a further embodiment is an alkyl-linked nucleotide non-homogeneous solid support comprising an alkyl-linked cytidine, said alkyl-linked nucleotide non-homogeneous solid support comprising the general structure:



10 or an ionized variant or a salt thereof.

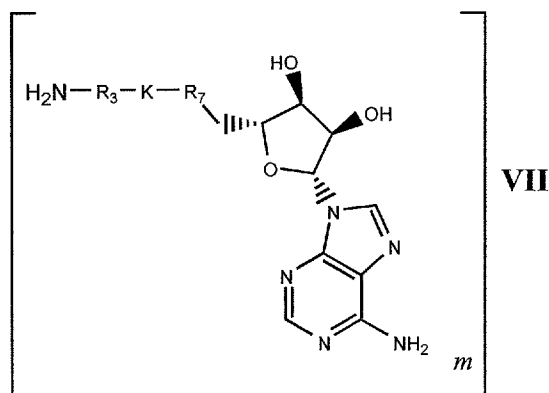
In yet another embodiment is an alkyl-linked nucleotide non-homogeneous solid support comprising an alkyl-linked uridine, said alkyl-linked nucleotide non-homogeneous solid support comprising the general structure:



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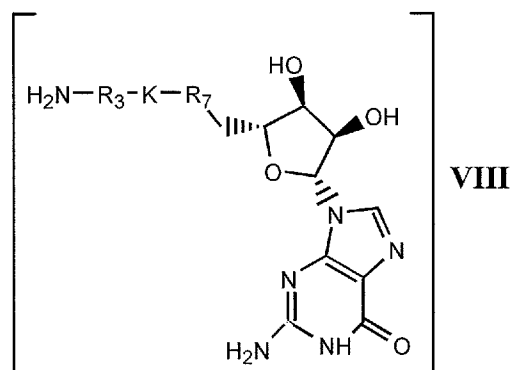
or an ionized variant or a salt thereof.

In another embodiment, the invention includes an alkyl-linked adenosine comprising the general structure:



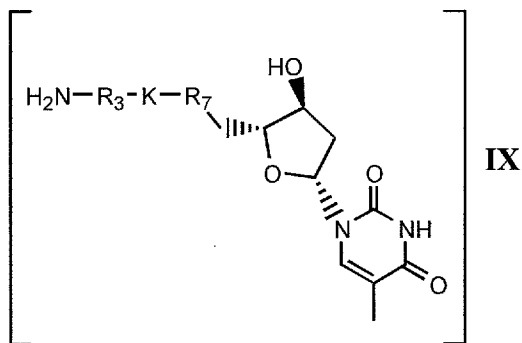
5 or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

In further embodiment, the invention includes an alkyl-linked guanosine, comprising the general structure:



15 or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

20 In another embodiment, the invention includes an alkyl-linked thymidine comprising the general structure:

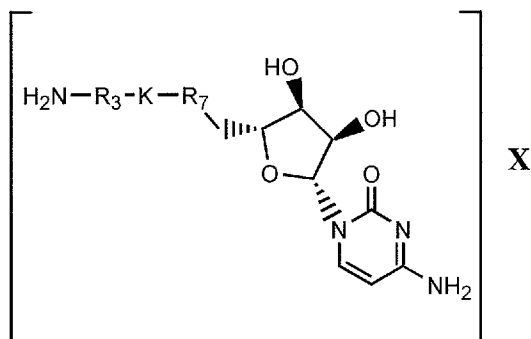


5

or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In yet another embodiment, the invention includes an alkyl-linked cytidine comprising the general structure:

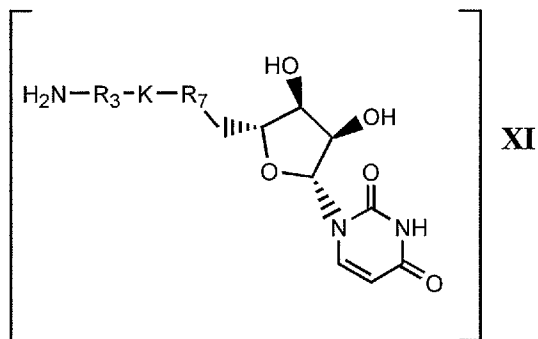


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or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

In a further embodiment, the invention includes an alkyl-linked uridine comprising the general structure:

20

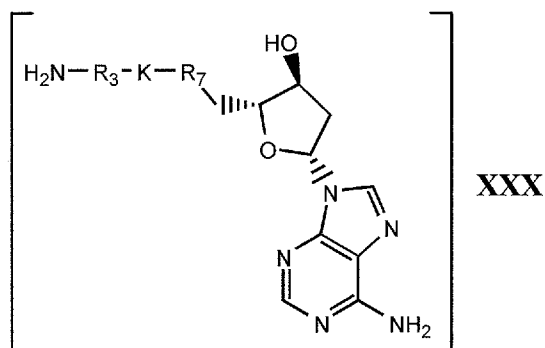


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or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In another embodiment, the invention includes an alkyl-linked 2'-deoxy-adenosine comprising the general structure:

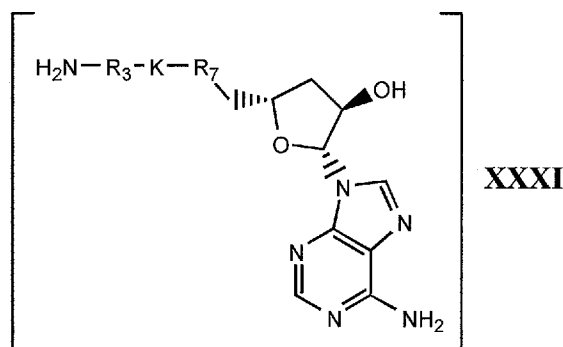


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or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

20

In another embodiment, the invention includes an alkyl-linked 3'-deoxy-adenosine comprising the general structure:

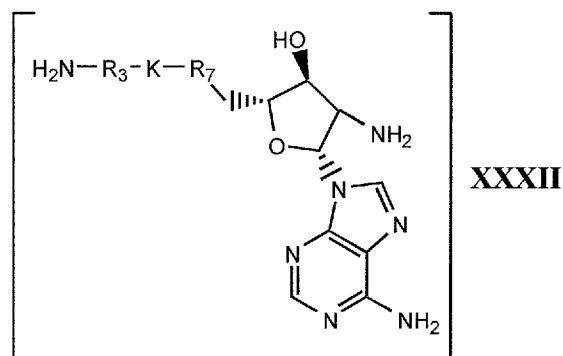


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or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In another embodiment, the invention includes an alkyl-linked 2'-deoxy-2'-amino-adenosine comprising the general structure:

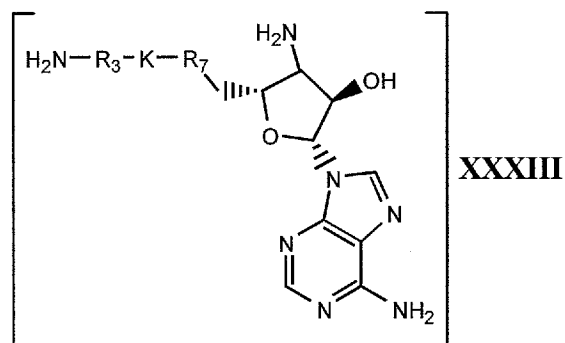


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or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

20

In another embodiment, the invention includes an alkyl-linked 3'-deoxy-3'-amino-adenosine comprising the general structure:

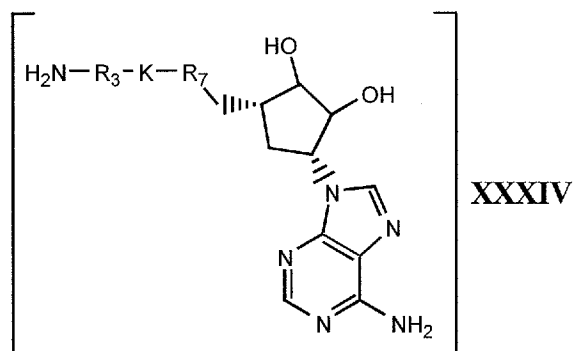


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or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In another embodiment, the invention includes an alkyl-linked adenosine derivative, Aristeromycin comprising the general structure:

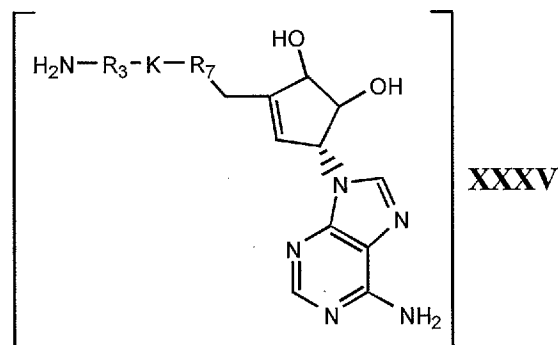


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or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

20

In another embodiment, the invention includes an alkyl-linked ATP derivative, Neplanocin A, comprising the general structure:

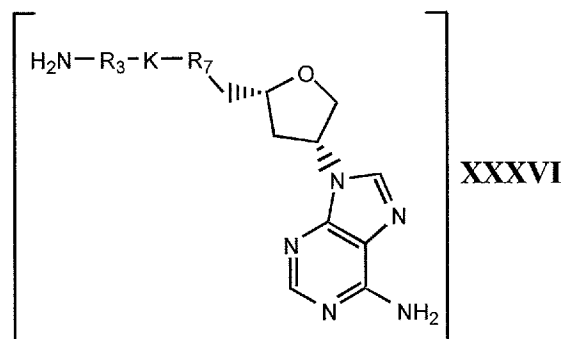


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or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

10

In another embodiment, the invention includes an alkyl-linked 2',3'-dideoxy-3'-oxoadenosine, comprising the general structure:



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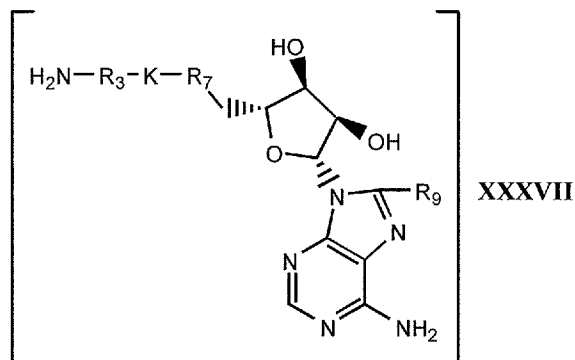
or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

20

In other embodiments, the invention includes an alkyl-linked 2-, 6-, or 8-substituted adenosine derivative. These substituted adenosine derivatives can be made by the condensation of the corresponding bromides (for the 2 and 8 position) or chlorides (for the 6 position) and the appropriate amine (including, for example, allylamine, benzylamine, t-butylamine, 2-methoxyethylamine, and diethylamine. *See, for example* Halbfinger *et al.* (1999) *J. Med. Chem.* 42:5325-37 and van Tilburg *et al.* (1999) *J. Med.*

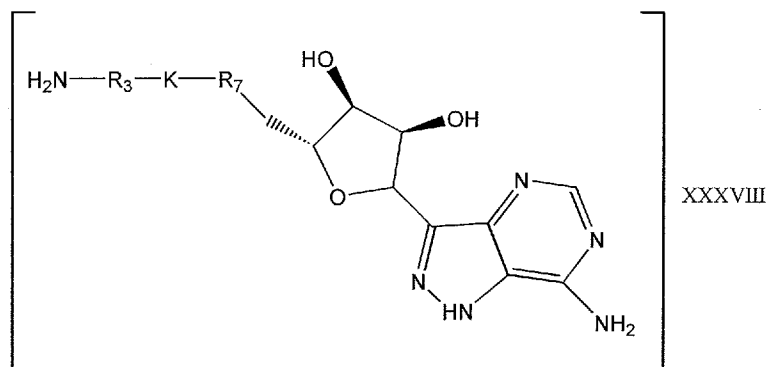


5 Chem. 42:1393-400; each of which is herein incorporated by reference in its entirety. An example of the general structure of alkyl -linked 8-substituted adenosine is shown below:



or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof, and  $R_9$  is an amine.

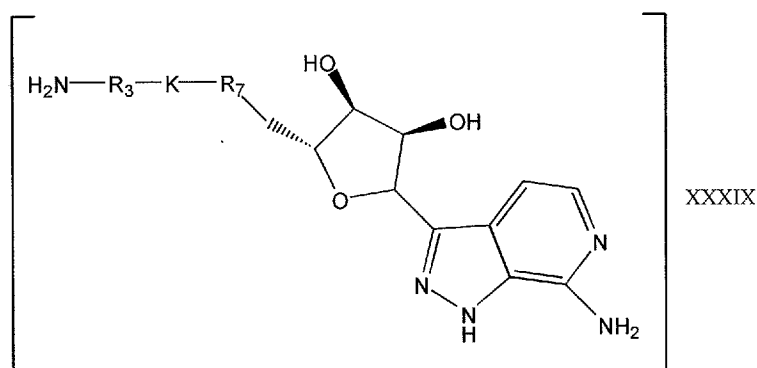
In an additional embodiment, the invention includes an alkyl-linked formycin A.  
15 An example of the general structure of alkyl -linked formycin A is shown below:



or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

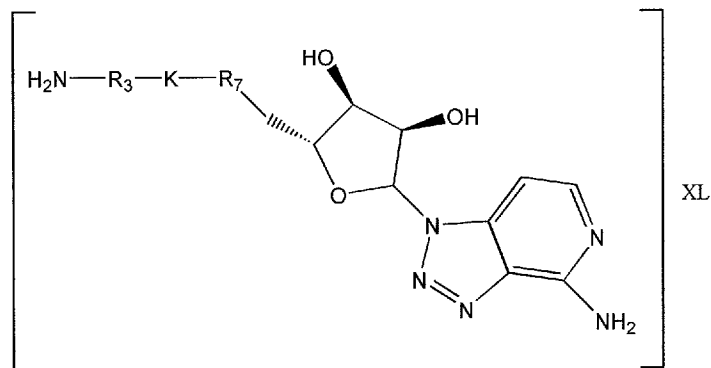
In an additional embodiment, the invention includes an alkyl-linked 4-deazaformycin. The synthesis of 4-deazaformycin A is described in Kourafalos *et al.*

5 (2003) *J. Org. Chem.* 68:6466-69, herein incorporated by reference. An example of the general structure of alkyl-linked 4-deazaformycin A is shown below:



or an ionized variant or a salt thereof, wherein the linker (R<sub>3</sub>) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted  
10 or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

In some embodiments, the invention includes an aza or deaza adenosine derivative. The synthesis of 8-aza, 8-aza-1-deaza, 8-aza-3-deaza, 1-deaza, 3-deaza, and  
15 1,7-deaza adenine derivatives is described in Franchetti *et al.* (1994) *J. Med. Chem.* 37, 3534 and Seela *et al.* (1991) *Helv. Chim. Acta* 74:1048. These derivatives can be reacted with sugar halides or  $\beta$ -D-ribofuranose-1-acetate-2,3,5-tribenzoate (*see*, Kraybill *et al.* (2002) *J. Am. Chem. Soc.* 124:12118 and Saneyoshi *et al.* (1979) *Chem. Pharm. Bull.* 27:2518) and SnCl<sub>4</sub> to form additional adenosine derivatives. An example of the general  
20 structure of one such derivative is shown below:

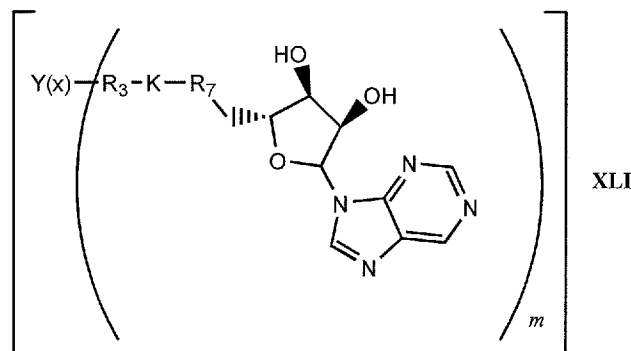


or an ionized variant or a salt thereof, wherein the linker (R<sub>3</sub>) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted

- 5 or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

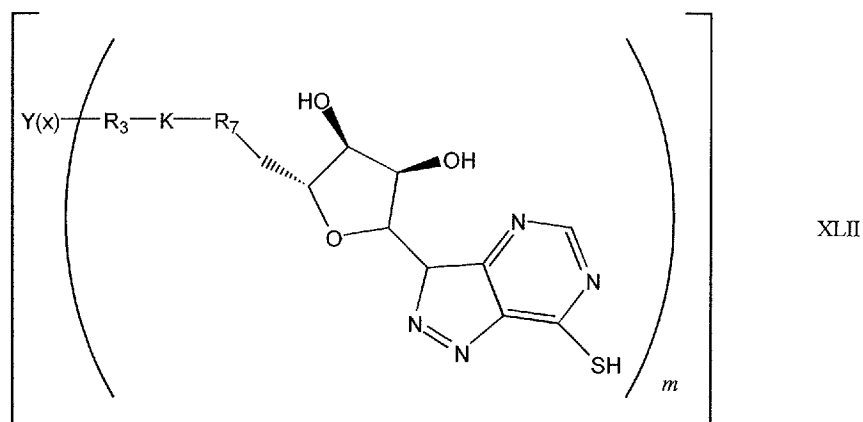
In a further embodiment, the invention includes an alkyl-linked purine riboside.

- 10 An example of the general structure of alkyl-linked purine riboside is shown below:



- or an ionized variant or a salt thereof, wherein the linker (R<sub>3</sub>) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.
- 15

The invention also provides an alkyl-linked 6-mercaptapurine riboside. An example of the general structure of alkyl-linked 6-mercaptapurine riboside is shown below:

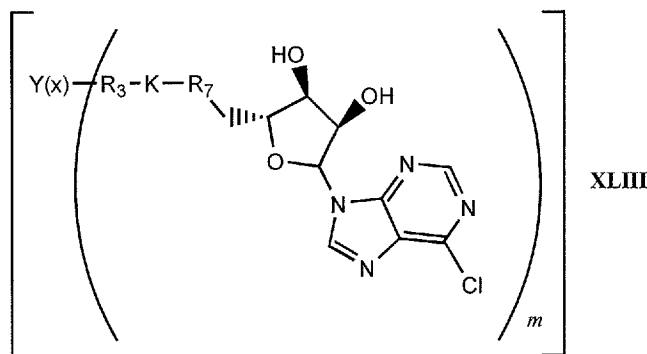


20

or an ionized variant or a salt thereof, wherein the linker (R<sub>3</sub>) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted

5 or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

The invention also provides an alkyl-linked 6-chloropurine riboside. An example of the general structure of alkyl-linked 6-chloropurine riboside is shown below:

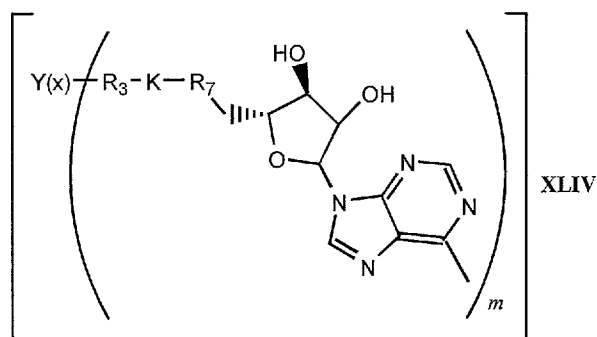


or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

15

The invention also provides an alkyl-linked 6-methyl purine riboside. The 6-methyl purine riboside may be synthesized by reacting 6-chloropurine riboside with the Grignard reagent methyl magnesium chloride to yield 6-methyl purine riboside (Hocék and Dvorakova (2003) *J. Org. Chem.* 68:5773-6, herein incorporated by reference). An example of the general structure of alkyl-linked 6-methyl purine riboside is shown below:

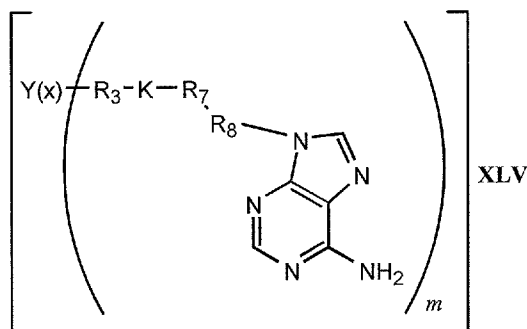
20



or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted

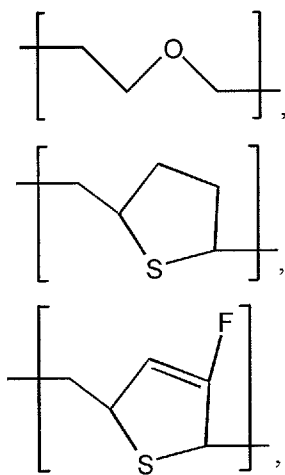
5 or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof.

In other embodiments, the invention includes an alkyl-linked adenosine derivative in which the ribose group has been replaced with a ribose mimic. One example of the  
10 general structure of such an alkyl-linked derivative is

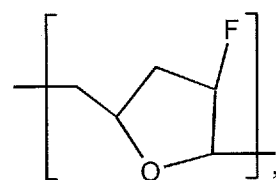
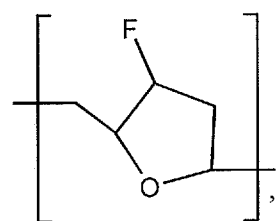
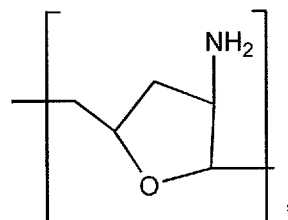
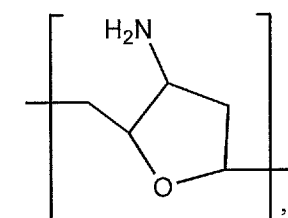
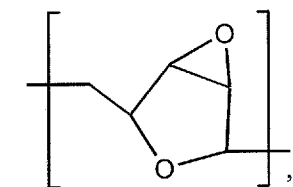
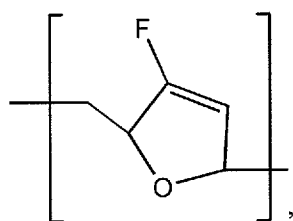
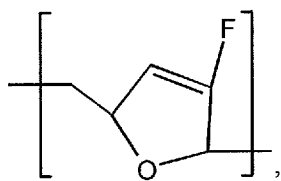


or an ionized variant or a salt thereof, wherein the linker ( $R_3$ ) is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted  
15 heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof and  $R_8$  is a ribose mimic. In some embodiments, the ribose mimic is an alkyl,  $C_4$ - $C_7$  cycloalkyl, heteroalkyl, aryl, or heteroaryl group. *See, for example Hernandez et al. (2002) J. Med. Chem. 45:4254-63, herein incorporated by reference in its entirety.*

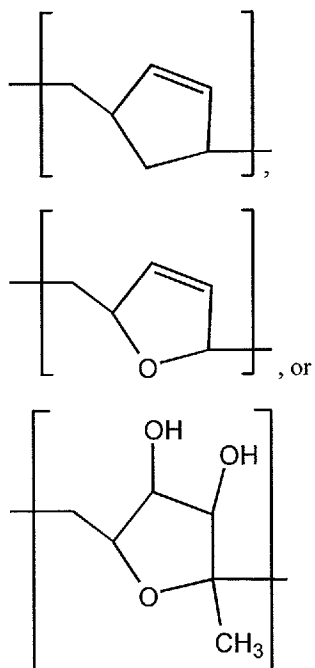
Examples of other suitable ribose mimics include, without limitation:



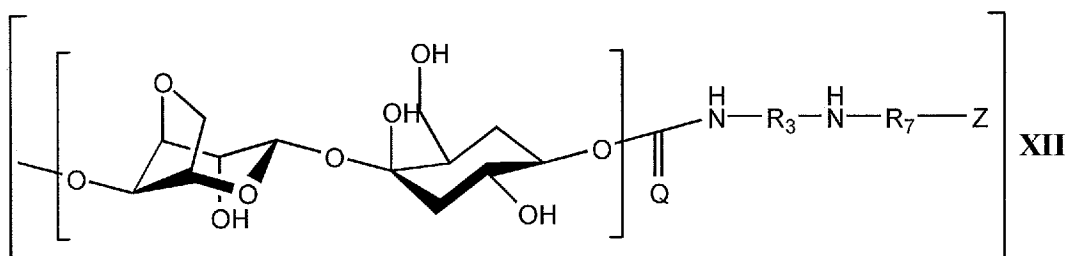
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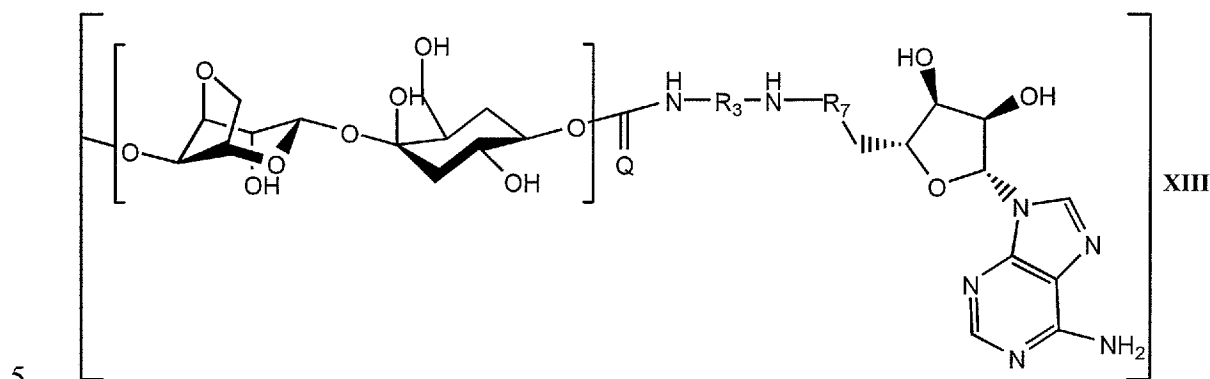


10 In another embodiment is an alkyl-linked nucleotide covalently bound to agarose comprising the general structure:



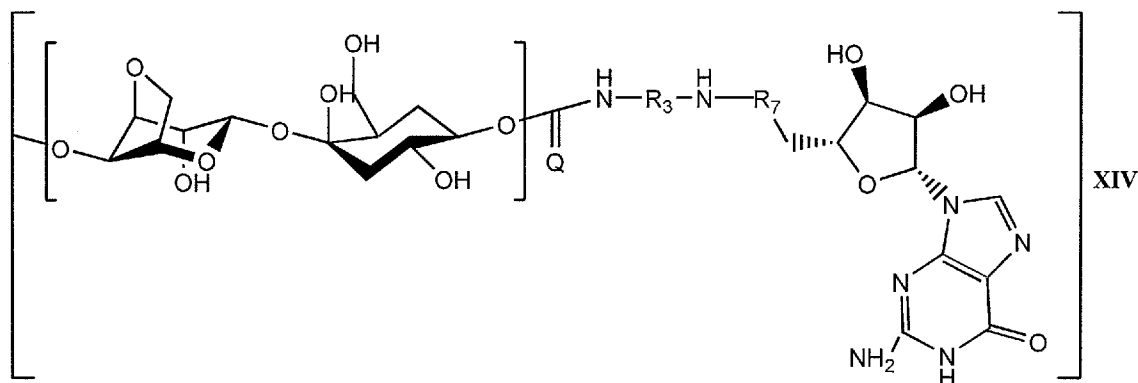
or an ionized variant or a salt thereof, wherein Q = NH<sub>2</sub><sup>+</sup> or O.

15 In one embodiment, the invention includes an alkyl-linked adenosine covalently bound to agarose comprising the general structure:



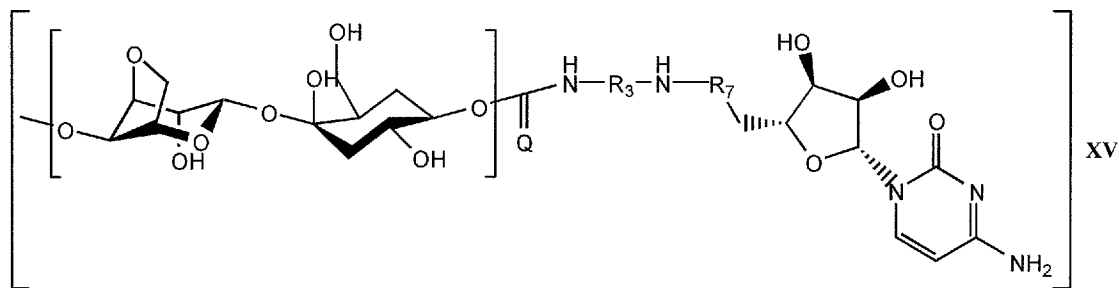
or an ionized variant or a salt thereof, wherein  $Q = \text{NH}_2^+$  or  $\text{O}$ .

In one embodiment, the invention includes an alkyl-linked guanosine covalently bound to agarose comprising the general structure:



10 or an ionized variant or a salt thereof, wherein  $Q = \text{NH}_2^+$  or  $\text{O}$ .

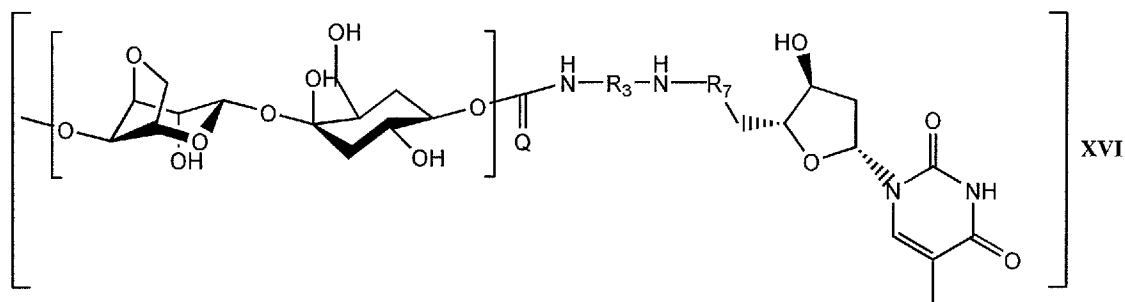
In one embodiment, the invention includes an alkyl-linked cytidine covalently bound to agarose comprising the general structure:



or an ionized variant or a salt thereof, wherein  $Q = \text{NH}_2^+$  or  $\text{O}$ .

15 In one embodiment, the invention includes an alkyl-linked thymidine covalently bound to agarose comprising the general structure:

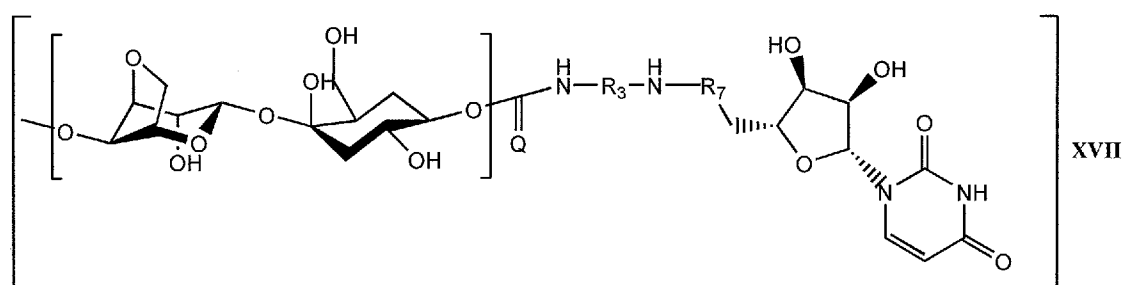




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or an ionized variant or a salt thereof, wherein Q = NH<sub>2</sub><sup>+</sup> or O.

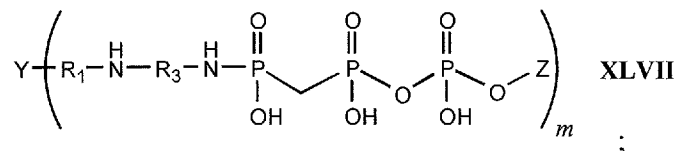
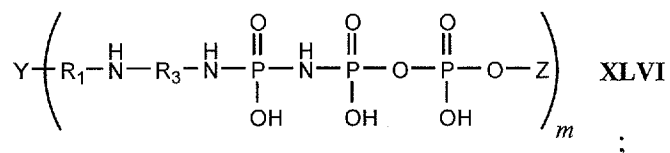
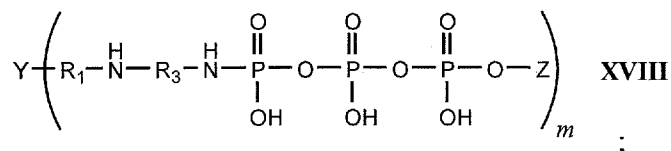
In one embodiment, the invention includes an alkyl-linked uridine covalently bound to agarose comprising the general structure:



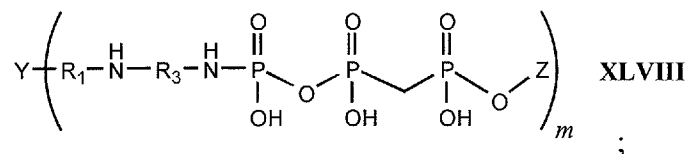
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or an ionized variant or a salt thereof, wherein Q = NH<sub>2</sub><sup>+</sup> or O.

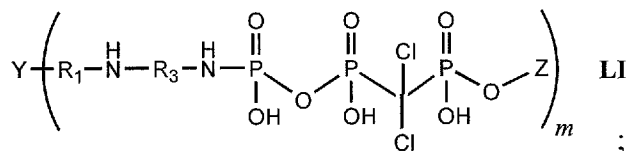
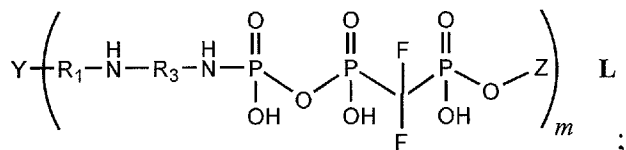
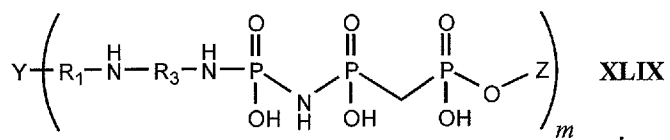
Also included in the invention are  $\gamma$ -alkyl-linked nucleotide triphosphates comprising the general formula:



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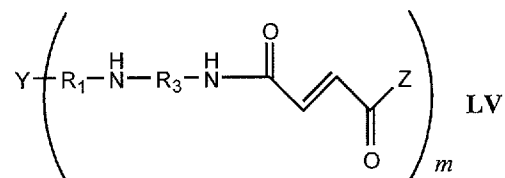
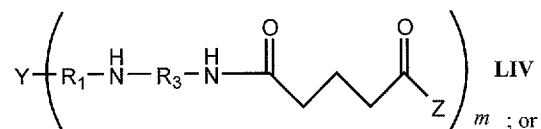
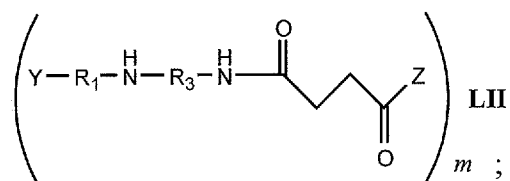
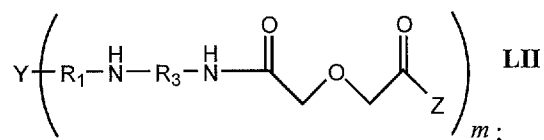
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or an ionized variant or a salt thereof.

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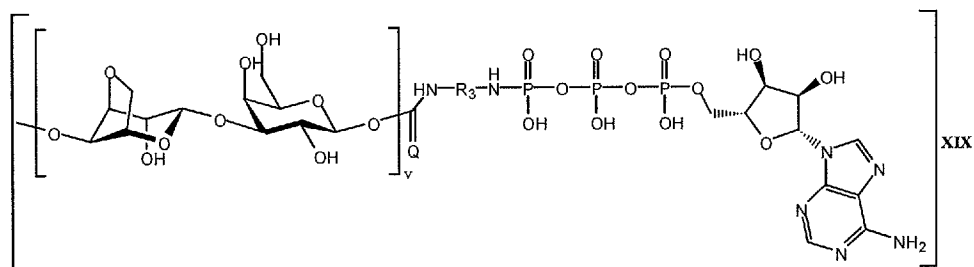
Also included in the invention are  $\gamma$ -alkyl-linked nucleotide analogs comprising the general formula:



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In one embodiment, the invention includes a  $\gamma$ -phosphate-linked adenosine triphosphate bound to an agarose solid support comprising the general formula:

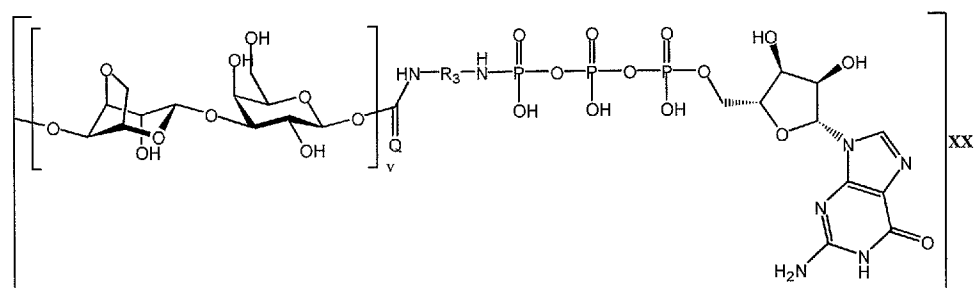
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or an ionized variant or a salt thereof.

In one embodiment, the invention includes a  $\gamma$ -phosphate-linked guanosine triphosphate bound to an agarose solid support comprising the general formula:

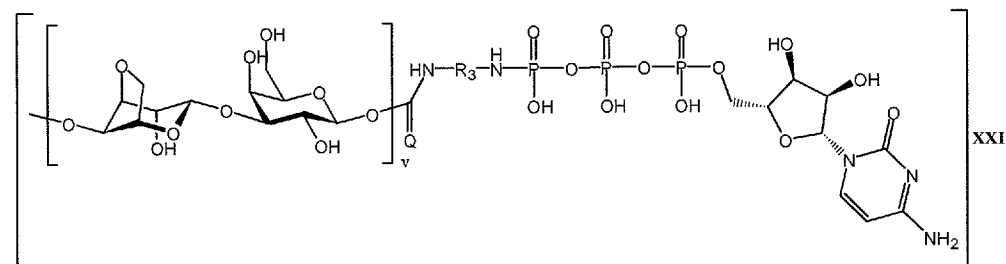
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or an ionized variant or a salt thereof.

In another embodiment, the invention includes a  $\gamma$ -phosphate-linked cytidine triphosphate bound to an agarose solid support comprising the general formula:

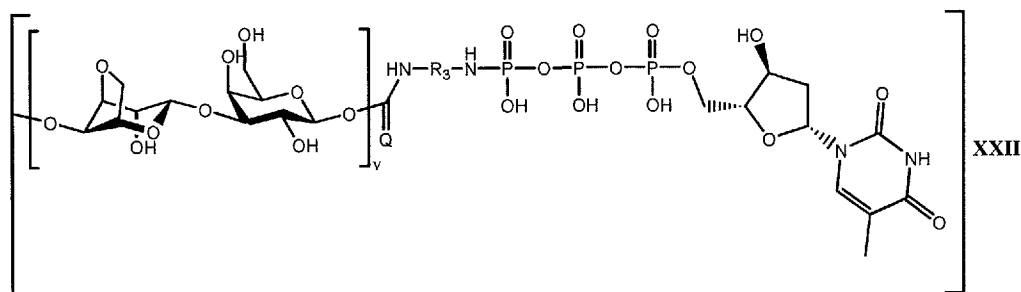
15



or an ionized variant or a salt thereof.

In a further embodiment, the invention includes a  $\gamma$ -phosphate-linked thymidine triphosphate bound to an agarose solid support comprising the general formula:

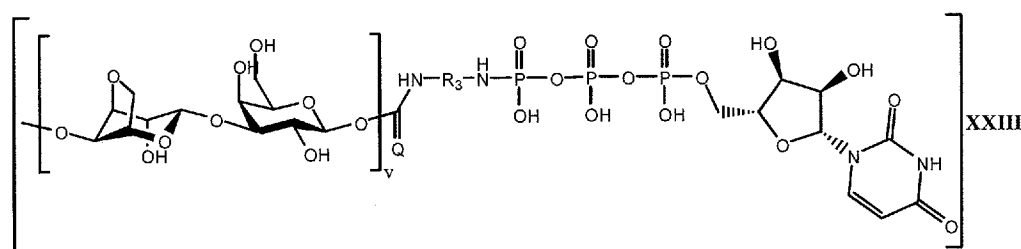
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or an ionized variant or a salt thereof.

In another embodiment, the invention includes a  $\gamma$ -phosphate-linked uridine triphosphate bound to an agarose solid support comprising the general formula:

10

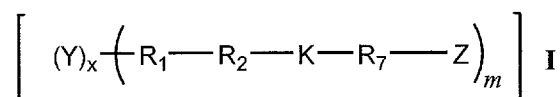


or an ionized variant or a salt thereof.

### Synthesis of Alkyl-linked Nucleotide Affinity Media

Also included in the invention is a method to synthesize an alkyl-linked nucleotide affinity medium comprising a general formula:

15



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comprising the general steps of (a) coupling at least one linker to a solid support or tag in a suitable coupling buffer, wherein the linker is  $R_2$ , or a combination of  $R_1$  and  $R_2$ ; (b) end-capping reactive sites remaining on the solid support or tag after the coupling step; and (c) reacting a terminal phosphate or thiophosphate group of a nucleotide with the linker coupled to the solid support or tag, wherein Y is a solid support or a tag;  $x = 1$ ;  $R_1$  is a covalent bond between Y and  $R_2$ , or  $R_1$  is an acyl group, a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof;  $R_2$  is a substituted or a non-substituted alkyl

25

5 group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof; K is a heteroatom; R<sub>7</sub> is (P)<sub>n</sub> where P is a phosphate or thiophosphate and n is at least one or R<sub>7</sub> is a phosphate group mimic, Z is a nucleoside or nucleoside derivative; and m is at least  
10 one. As described elsewhere herein, a solid support can be any suitable support, such as a resin, or a particulate material, such as a bead, or a particle. Alternatively, a solid support can be a continuous solid surface, such as a plate, chip, well, channel, column or a tube. The material of a solid support will be of any suitable substance, compound or polymer, as will be appreciated by one of skill in the art. Examples include, without  
15 limitation, acrylamide, agarose, methacrylate polymers, methacrylate co-polymers, thermoresponsive polymers, cellulose, nylon, silica, glass, ceramic, a magnetized particle or surface, nitrocellulose, polystyrene and derivatives thereof.

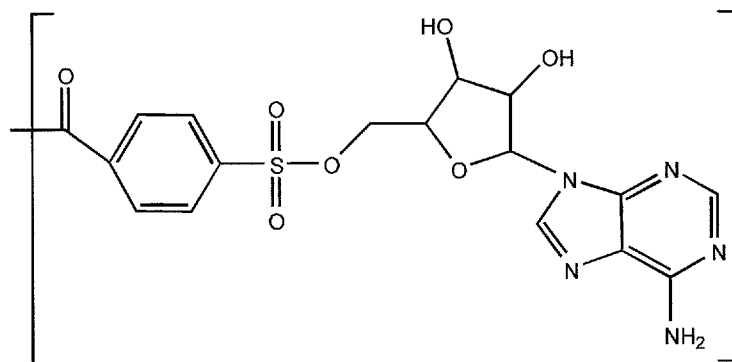
Furthermore, a tag, as used herein, is an agent that provides for the specific detection or capture of the alkyl-linked nucleotide. For example, and without limitation,  
20 a suitable tag is biotin, avidin, streptavidin, a hapten, a fluorophore or a chromophore. Detection or capture of the alkyl-linked nucleotide employs techniques that are standard in the art. For example, a biotin-tagged alkyl-linked nucleotide can be captured using avidin, streptavidin or related avidin derivatives. The biotin-avidin interaction is highly specific. An avidin-conjugated agent used to detect or capture a biotin-tagged alkyl-  
25 linked nucleotide can be soluble (for example, an antibody), or a particulate material (for example, beaded agarose or a magnetized particle), or a continuous surface (for example, a plate or well coated with avidin). Detection and capture of a hapten-tagged alkyl-linked nucleotide can be achieved, for example, using hapten-specific antibodies. Visual  
30 detection methods for fluorophore or chromophore-tagged alkyl-linked nucleotides are readily understood by one of skill in the art.

A nucleoside and a nucleotide, as referred to herein, can be naturally-occurring or non-naturally-occurring. Furthermore, the nucleoside or nucleotide can be biologically or synthetically-derived using techniques that are standard to one of skill in the art. Suitable nucleosides include, without limitation, adenosine (A), guanosine (G), cytidine (C),  
35 thymidine (T) and uridine (U), and derivatives thereof.

5 In one embodiment, the nucleotide is a monophosphate, diphosphate, triphosphate, or tetraphosphate of adenosine, guanosine, cytidine, thymidine, or uridine or an analog thereof. In certain embodiments, the phosphate moiety of the nucleotide is modified. See, for example Picher *et al.* (1996) *Biochem. Pharmacol.* 51:1453-60; Ingall  
10 *et al.* (1999) *J. Med. Chem.* 42:213-20; Gendron *et al.* (2000) *J. Med. Chem.* 43:2239-47; and Xu *et al.* (2002) *J. Med. Chem.* 45:5694-709 each of which is herein incorporated in its entirety by reference.

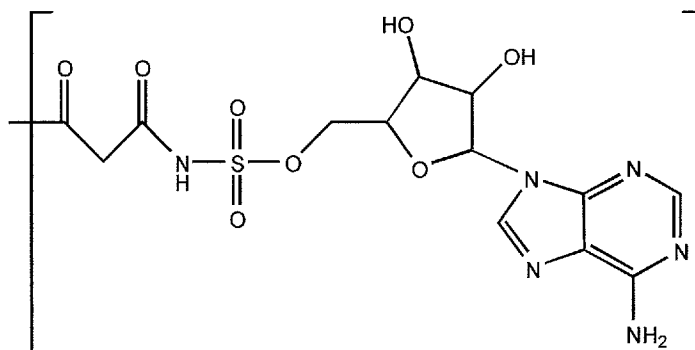
In other embodiments, the phosphate moiety of the nucleotide is replaced by a phosphate group mimic. For example, in some embodiments, the alkyl-linked nucleotide non-homogeneous solid supports comprise a carboxylic acid that contains 4-8 carbons in  
15 the main chain and optionally contains a heteroatom. To make these nucleotide derivatives, 2',3'-isopropylidene adenosine is reacted with a cyclic anhydride, and then the acetonide is deprotected. Examples of cyclic anhydrides that may be reacted with 2',3'-isopropylidene include diglycolic anhydride, succinic anhydride, glutaric anhydride, and maleic anhydride, although any suitable electrophile may be used. The functionality of  
20 the 5'-hydroxyl of adenosine is may also be converted into a primary amine by a Mitsunobu reaction with phthalimide followed by hydrazinolysis. See, for example Bressi *et al.* (2001) *J. Med. Chem.* 44:2080-93 and Herforth *et al.* (2002) *J. Comb. Chem.* 4:302-14; each of which are herein incorporated by reference in their entirety.

Other phosphate group mimics may be used according to invention. For example,  
25 an ATP analog can be made by reacting 2',3'-O-isopropylidene-adenosine with 4-chlorosulfonylbenzoic acid to give an ATP analog having the formula:



The benzene ring can be substituted with hydroxyl or amine groups. See, for example, Rosse *et al.* (1997) *Helv. Chim. Acta.* 80:653, herein incorporated by reference.

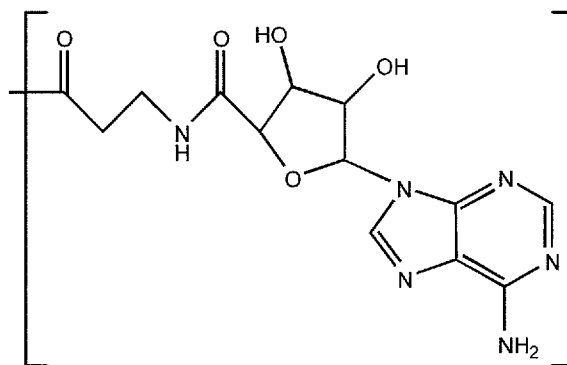
5            In another example, 2',3'-O-isopropylidene-adenosine is reacted with sulfamoyl chloride, the sulfonamide is conjugated with a carboxylic acid (for example, benzyl malonate or *t*-butyl malonate) and then deprotected in one or two steps to give an ATP analog having the structure:



10

After conjugation with *t*-butyl malonate only one acidic deprotection step is performed; After conjugation with benzyl malonate a catalytic hydrogenation of the benzyl group and an acidic cleavage of the acetonide group are performed. In addition to mono-protected malonates, mono-protected succinates or glutarates can be used. In addition, 15 the sulfonamide can be reacted with other electrophiles such as bromoacetic acid to obtain phosphate group mimics. Amino acids can also be used to link the sulfonamide with a linker. See, Koroniak *et al.* (2003) *Pharmacology & Therapeutics* 93:145, herein incorporated by reference.

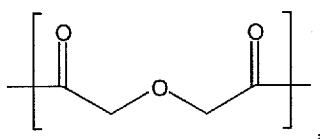
20            In a further example, an ATP analog is made by conjugating adenosine-5'-carboxylic acid with  $\beta$ -alanine to form an ATP analog having the structure:



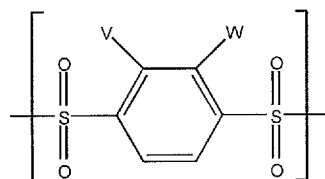
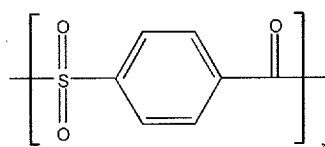
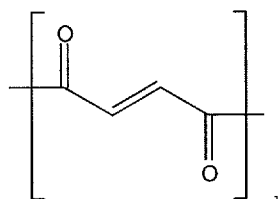
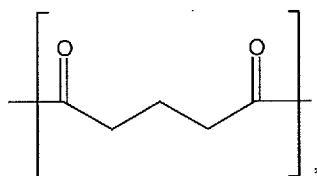
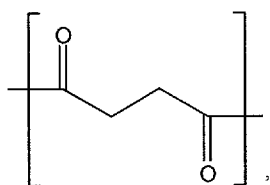
Other aminocarboxylic acids can be used. In addition, adenosine-5'-carboxylic acid can be conjugated with amino acids or peptides. See, Loog *et al.* (2000) *FEBS Letters*

5 480:244; and Parang *et al.* (2002) *Pharmacology and Therapeutics* 93:145, both of which are herein incorporated by reference in their entirety.

Other non-limiting examples of phosphate group mimics that may be used in the alkyl-linked nucleotide non-homogeneous solid supports of the present invention include:

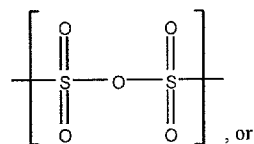


10

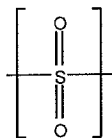


where V and W can be H or a heteroatom such as NH<sub>2</sub> or OH,

15







The ribose moiety of the alkyl-linked nucleotide may be modified or replaced. For example, oxidation and acetonide protection of 4-penten-1-ol yields an intermediate that can be reacted with the adenine under classic Mitsunobu conditions. Mitsunobu reactions between alcohols and the adenine N9 are well established. *See*, for example, Chang *et al.* (1999) *Chemistry & Biology* 6:361-75. Deprotection of the acetonide then leads to a first ligand ready for phosphorylation and attachment to a resin-linker arm combination. The primary alcohol of the same intermediate can also be oxidized to the aldehyde, followed by acetonide deprotection and ring closure. Selective protection of the primary hydroxyl over the secondary hydroxyl, a Mitsunobu reaction, and deprotection of the primary hydroxyl results in a second ligand.

Non-limiting examples of nucleoside analogs that may be used in the compositions of the invention include 2'-deoxy-adenosine, 3'-deoxy-adenosine, 2'-deoxy-2'-amino-adenosine, 3'-deoxy-3'-amino adenosine, formycin A, 4-deazaformycin A, aristeromycin, neplanocin A, purine riboside, 6-mercaptopurine riboside, 6-chloropurine riboside, 6-methyl purine riboside, and 2',3'-dideoxy-3'-oxoadenosine. *See*, for example, Guranowski *et al.* (1981) *Biochemistry* 20:110; Yaginuma *et al.* (1981) *J. Antibiot.* 23:359; Robins *et al.* (1983) *J. Am. Chem. Soc.* 105:4059; Borchardt *et al.* (1984) *J. Biol. Chem.* 259:5353; De Clerq *et al.* (1987) *Biochem. Pharmacol.* 36:2567; Huryn *et al.* (1989) *Tetrahedron Lett.* 30:6259; Franchetti *et al.* (1994) *J. Med. Chem.* 37:3534; Van Calenbergh *et al.* (1994) *Helv. Chim. Acta.* 77:631; Cowart *et al.* (1999) *J. Org. Chem.* 64:2240; Hock and Dvorakova (2003) *J. Org. Chem.* 68:5773; and Kourafalos *et al.* (2003) *J. Org. Chem.* 68:6466-69; each of which is herein encompassed by reference in its entirety.

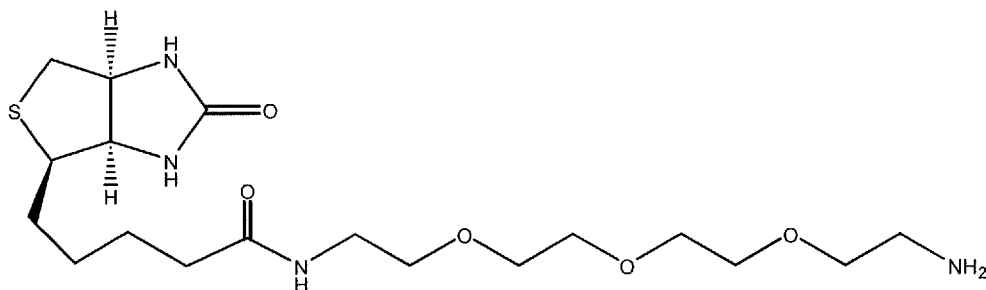
In still other embodiments, the nucleoside or nucleotide is a 2-, 6- or 8-substituted adenosine derivative. Such derivatives may be made by the condensation of the corresponding bromides (for the 2 and 8 position) or chlorides (for the 6 position) and the appropriate amine, *e.g.* allylamine, benzylamine, *t*-butylamine, 2-

5 methoxyethylamine, diethylamine. See, for example Halbfinger *et al.* (1999) *J. Med. Chem.* 42: 5325, and van Tilburg *et al.* (1999) *J. Med. Chem.* 42:1393.

In some embodiments, the compositions comprise a ribose mimic. For example, the ribose of the nucleoside or nucleotide is replaced with an alkyl, C<sub>4</sub>-C<sub>7</sub> cycloalkyl, heteroalkyl, aryl, or heteroaryl group. This may be accomplished by monotitrylation of diols, followed by a Mitsunobu reaction with adenine and deprotection of the titrylated alcohol. See, for example, Hernandez *et al.* (2002) *J. Med. Chem.* 45, 4254, herein incorporated by reference. Other ribose mimics and their syntheses are described, for example, in Lee *et al.* (1961) *J. Am. Chem. Soc.* 83:1906; Imazawa (1978) *J. Org. Chem.* 43:3044; Robins *et al.* (1984) *Tetrahedron Letters* 25:367; Herdewijn *et al.* (1987) *J. Med. Chem.* 30:2131-37; Wu *et al.* (1988) *Tetrahedron* 44:6705; Van Aerschot *et al.* (1989) *J. Med. Chem.* 32:1743-49; Secrist *et al.* (1991) *J. Med. Chem.* 34:2361-66; Secrist *et al.* (1992) *J. Med. Chem.* 35:533-38; Holletz *et al.* (1994) *Synthesis* 8:789; Choi *et al.* (1998) *Tetrahedron Letters* 25:367; Meier *et al.* (1999) *Nucleosides Nucleotides* 18:907-12; Meier *et al.* (1999) *J. Med. Chem.* 42:1615-24; and Choo *et al.* (2003) *J. Med. Chem.* 46:389-98.

The synthesis of an alkyl-linked nucleotide affinity medium comprising alkyl-linked nucleotides is generally done in three steps: first the linker (or linkers) is attached to the solid support (also referred to herein generally as a resin); second, any remaining active sites on the solid support are end-capped using a suitable reagent such as, for example, ethanolamine, 2-amino-2-(hydroxymethyl)-1,3-propanediol, or glycine; and third, the linker arm is reacted with the affinity ligand of choice, for example, a nucleotide, such as, adenosine triphosphate (ATP), thus producing a nucleotide affinity medium.

The attachment of a tag, such as biotin, to a nucleotide via a linker uses techniques that are standard in the art. For example, biotin can be synthesized with a linker arm attached; such compounds are known to one of skill in the art and are commercially available with different linker arms already attached; for example:



To synthesize an alkyl-linked nucleotide attached to a biotin tag, the water-soluble biotin linker complex is reacted with the nucleotide which has been reacted with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-methyl imidazole.

The synthesis of an alkyl-linked nucleotide with a protective group uses techniques that are known in the art. Typically the linker, for example a diamine linker, is asymmetrically protected (i.e. protected at one end) to form a water-soluble semi-protected diamine linker. The unprotected end of the linker, is reacted with a nucleotide which has been prepared by reacting the nucleotide with EDC and 1-methyl imidazole, as will be understood by one of ordinary skill in the art.

As already described herein, an alkyl-linked nucleotide attached to a solid support or a tag, such that the solid support or tag is suitable for the separation of the alkyl-linked nucleotide, and optionally, compounds (such as proteins, for example) bound to the alkyl-linked nucleotide, from unbound compounds, is also referred to herein as a “nucleotide affinity medium or media”, or as an “alkyl-linked nucleotide affinity medium or media.”

Generally, the linker is attached to the solid support in any suitable coupling buffer as will be understood by one of skill in the art. For example, the coupling buffer can be 0.1M or 0.2M sodium phosphate, pH adjusted to 8-9 for cyanogen bromide-activated SEPHAROSE™, or 0.1M or 0.2M sodium phosphate, pH adjusted to 10 for 1,1'-carbonyl diimidazole-activated (CDI)- SEPHAROSE™. Alternatively, 0.01M to 0.1M borate, with pH adjusted to 8-9 can be used for coupling cyanogen bromide-activated SEPHAROSE™, or 0.01M to 0.1M borate, with pH adjusted to 10 can be used for coupling CDI-activated SEPHAROSE™. For example, a linker can be reacted at room temperature with cyanogen bromide-activated SEPHAROSE™ beaded agarose in a sodium bicarbonate coupling buffer (for example, and as used in the Examples below,

5 0.1 M NaHCO<sub>3</sub>, 0.5 M NaCl, pH = 8.2). For reactions with CDI-activated cross-linked SEPHAROSE™ beaded agarose, the linker can be, for example, reacted at room temperature with the resin in a 0.05 M NaHCO<sub>3</sub>-Na<sub>2</sub>CO<sub>3</sub> coupling buffer, pH = 10, such as will be understood by one of skill in the art. Typically, the solid support is then washed with water.

10 The solid support is then end-capped to block remaining active sites on the solid support and is a standard technique that can be performed with any suitable reagent, such as, ethanolamine, 2-amino-2-(hydroxymethyl)-1,3-propanediol, or glycine. To illustrate, end-capping can be achieved by reacting the solid support with 1M ethanolamine (pH = 8.9) for approximately 1 hour at room temperature. The solid support is then typically  
15 washed with 1M NaCl and water.

The nucleotide is then reacted with the linker arm on the solid support under suitable conditions, as will be understood by one of skill in the art, and as described in the Examples.

Once prepared, an alkyl-linked nucleotide bound to a solid support or tag  
20 (nucleotide affinity medium) is stored in any suitable buffer. For example, 0.1M K<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub> buffer (pH = 7.4), containing 0.02% sodium azide as a preservative.

Synthesis methods for isourea and carbamate linkages are standard in the art (see generally, Hermanson *et al.*, "Immobilized Affinity Ligand Techniques", Academic Press, 1992, the teaching of which is incorporated herein by reference in its entirety). For  
25 example, the use of a cyanogen bromide-activated agarose (see, generally, Cuatrecasas and Anfinsen, "Affinity Chromatography" in Ann. Rev. Biochem. Snell et al., eds. (CA: Annual Reviews Inc.), 40: 259-278 (1971), the teachings of which are incorporated herein by reference in their entirety) provides for the synthesis of a suitable isourea linkage, whereas the use of a CDI-activated cross-linked SEPHAROSE™ beaded agarose  
30 6B (Pierce Biotechnology, Inc.) provides a suitable carbamate linkage. Alternatively, the hydroxyl group of a suitable resin can be converted into suitable leaving groups using N,N'-disuccinimidylcarbonate as an intermediate to prepare a carbamate linkage, or using organic sulfonyl chlorides to activate the resin hydroxyl groups for nucleophilic displacement to prepare a carbon-nitrogen bond.

5           The alkyl-linked nucleotides bound to a solid support in the following  
exemplification were made with cyanogen bromide-activated SEPHAROSE™ beaded  
agarose 4B (Sigma), CDI-activated cross-linked SEPHAROSE™ beaded agarose 6B  
(Pierce Biotechnology, Inc.), TOYOPEARL® resins, SEPHACRYL™ resins, Trisacryl  
10   resins, or Ultrogel resins. Other suitable solid supports will be readily appreciated by one  
of skill in the art and include, for example and without limitation, acrylamide, agarose,  
methacrylate polymer, methacrylate copolymer, cellulose, nylon, silica, magnetized  
particle, nitrocellulose and polystyrene, and derivatives thereof.

          Prior to use, cyanogen bromide-activated SEPHAROSE™ beaded agarose is  
washed with 1mM HCl and water, whereas CDI-activated cross-linked SEPHAROSE™  
15   beaded agarose is washed with ice-cold water, in accordance with standard protocols, as  
will be understood by one of skill in the art.

          One example of the chemical synthesis of a  $\gamma$ -phosphate-linked nucleotide affinity  
ligand is illustrated in Figs. 1-5. For the purpose of illustration, the nucleotide is ATP.

          Figure 1 illustrates the chemical formation of Intermediate IA. Cyanogen  
20   bromide-activated SEPHAROSE™ beaded agarose is reacted with a diamino-  
hydrophobic linker to form a resin-bound linker (Intermediate IA).

          Using an alternative resin, Figure 2 illustrates the chemical formation of  
Intermediate IB. CDI-activated SEPHAROSE™ beaded agarose is reacted with a  
diamino-hydrophobic linker to form a resin-bound linker (Intermediate IB).

25           The chemical formation of Intermediate II, which is the modification of the  
nucleotide in preparation of its attachment to the resin, is illustrated in Figure 3. The  
nucleotide is reacted with a water-soluble carbodiimide, such as 1-(3-  
dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC) to form O-phosphoryl  
isourea. The O-phosphoryl isourea is then reacted with a suitable nucleophilic  
30   compound, for example, 1-methyl imidazole to form Intermediate II.

          The final steps for the synthesis of nucleotide affinity media are illustrated in  
Figures 4 and 5. Specifically, Fig. 4 illustrates one example of the chemical formation of  
a nucleotide affinity medium. Intermediates IA and II are combined to form the final  
alkyl-linked nucleotide bound to a solid support. In another example, shown in Figure 5,

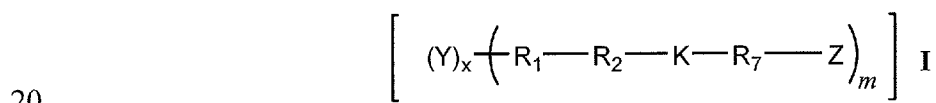
5 Intermediates IB and II are combined to form the final alkyl-linked nucleotide bound to a solid support.

In one embodiment, the loading of a solid support with an alkyl-linked nucleotide can be varied. This means that not necessarily all reactive sites on a solid support are reacted with an alkyl-linked nucleotide. For example, the loading of a solid support with  
10 an alkyl-linked nucleotide can be in a range of 5-25%, meaning 5-25% of reactive sites are reacted with an alkyl-linked nucleotide. Alternatively, the loading of the alkyl-linked nucleotide is in a range of 20-50%, 40-65%, 60-80% or 75-100%. The reactive groups on the solid support which are not reacted with an alkyl-linked nucleotide can be capped using a suitable reagent as appropriate.

15

### Utility of Alkyl-linked Nucleotide Compositions

Also included in the invention is a method for screening compounds, for example, with a proteome comprising the steps of (a) contacting a proteome with a nucleotide affinity medium comprising a general formula:



wherein Y is a solid support or a tag;  $x = 1$ ;  $R_1$  is a covalent bond between Y and  $R_2$ , or  $R_1$  is an acyl group, a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl group, a substituted or a non-substituted  
25 aryl group, a substituted or a non-substituted heteroaryl group, or a combination thereof;  $R_2$  is a substituted or a non-substituted alkyl group, a substituted or a non-substituted cycloalkyl group, a substituted or a non-substituted heteroalkyl group, a substituted or a non-substituted heterocycloalkyl, a substituted or a non-substituted heteroaryl group, or a combination thereof; K is a heteroatom;  $R_7$  is  $(P)_n$  where P is a phosphate or  
30 thiophosphate and n is at least one or  $R_7$  is a phosphate group mimic, Z is a nucleoside or nucleoside derivative; and m is at least one; (b) washing the nucleotide affinity medium with a buffer, whereby non-specifically bound components of the proteome are eluted from the nucleotide affinity medium and specific components of the proteome remain bound to the nucleotide affinity medium; (c) contacting the nucleotide affinity medium

5 bound to specific components of the proteome with at least one test compound; (d) eluting from the nucleotide affinity medium components of the proteome that are specifically displaced by the test compound; and (e) identifying the components of the proteome that are specifically displaced by the test compound from the nucleotide affinity medium.

10 A test compound can be any compound that is organic or inorganic, naturally-occurring or non-naturally occurring, as will be appreciated by one of skill in the art. For example, the test compound can be a compound from a combinatorial library or a chemical library. Furthermore, the test compound can be a compound extracted from a single cellular organism, a multicellular organism, or from an organ or a tissue of a  
15 multicellular organism. Examples of such organisms include, without limitation, bacteria, algae, fungi, plant, fish, amphibians, mammals, and the like. The test compound can be a single compound, or alternatively, a mixture of compounds, as will be understood by one of skill in the art.

As described elsewhere herein, a solid support can be any suitable support, such  
20 as a resin, or a particulate material, such as a bead, or a particle. Alternatively, a solid support can be a continuous solid surface, such as a plate, chip, well, channel, column or a tube. The material of a solid support will be of any suitable substance, compound or polymer, as will be appreciated by one of skill in the art. Examples include, without limitation, acrylamide, agarose, methacrylate polymer, methacrylate copolymer,  
25 cellulose, nylon, silica, glass, ceramic, a magnetized particle or surface, nitrocellulose, polystyrene and derivatives thereof.

Furthermore, a "tag", as that term is employed herein, is an agent that provides for the specific detection or capture of the alkyl-linked nucleotide. For example, and without limitation, a suitable tag is biotin, avidin, streptavidin, a hapten, a fluorophore or a  
30 chromophore. Detection or capture of the alkyl-linked nucleotide employs techniques that are standard in the art. For example, a biotin-tagged alkyl-linked nucleotide can be captured using avidin, streptavidin or related avidin derivatives. The biotin-avidin interaction is highly specific. An avidin-conjugated agent used to detect or capture a biotin-tagged alkyl-linked nucleotide can be soluble (for example, an antibody), or a  
35 particulate material (for example, beaded agarose or a magnetized particle), or a

5 continuous surface (for example, a plate or well coated with avidin). Detection and capture of a hapten-tagged alkyl-linked nucleotide can be achieved, for example, using hapten-specific antibodies. Visual detection methods for fluorophore or chromophore-tagged alkyl-linked nucleotides are readily understood by one of skill in the art. The visual detection of fluorophore or chromophore-tagged alkyl-linked nucleotides will be  
10 useful for techniques such as rapidly determining the presence or absence of a specific interaction between the tagged alkyl-linked nucleotide and a target protein. Alternatively, the visual detection of fluorophore or chromophore-tagged alkyl-linked nucleotides will be useful for detecting the presence or absence of a specific interaction between a test compound and a tagged alkyl-linked nucleotide bound to a target protein.  
15 Furthermore, linker-specific effects on the affinity or avidity of the affinity ligand for a compound can be monitored using fluorophore or chromophore-tagged alkyl-linked nucleotides. For example, a linker can affect the selectivity, affinity or avidity of the alkyl-linked nucleotide for an interacting compound via steric hindrance or electrostatic interactions, for example. These linker-specific effects can be assayed or monitored by  
20 visually detecting which linkers attached to a nucleotide specifically affect the interaction of the nucleotide with the target protein or compound. Such linkers can be used to prepare nucleotide affinity media that selectively bind a subset of proteins or target compounds.

As described elsewhere herein, a nucleoside and a nucleotide, as referred to  
25 herein, can be naturally-occurring or non-naturally-occurring. Furthermore, the nucleoside or nucleotide can be biologically or synthetically-derived using techniques that are standard to one of skill in the art. Suitable nucleosides include, without limitation, adenosine (A), guanosine (G), cytidine (C), thymidine (T) and uridine (U).

In one embodiment, the nucleotide is a monophosphate, diphosphate, or  
30 triphosphate of adenosine, guanosine, cytidine, thymidine, or uridine or an analog thereof.

The alkyl-linked nucleotide affinity media of the invention can be used, for example, in affinity chromatography techniques using methods that are known to one of skill in the art. See, for example, WO 00/63694, filed 12 April, 2000 and U.S. Patent No.



5 5,536,822, filed March 4, 1994, the teachings of which are incorporated herein by reference in their entirety.

The nucleotide affinity media and alkyl-linked nucleotides are useful for the detection and purification of biological compounds that bind to a nucleotide. For example,  $\gamma$ -phosphate-linked adenosine triphosphate (ATP) can be used to detect and  
10 purify biological compounds such as kinases, which are known to bind ATP. Specifically, an alkyl-linked nucleotide, such as ATP, can be bound to a solid support or tag, and subsequently contacted with, or mixed with, a proteome or part thereof. Non-specifically-interacting components of a proteome are generally removed by washing with a suitable buffer, as will be readily understood by one of skill in the art.

15 Additionally, the alkyl-linked nucleotide can be used to screen chemical compounds that specifically interact with a protein captured by the alkyl-linked nucleotide. Subsequently, this protein can be identified using art-standard techniques.

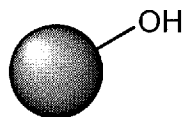
Alternatively, the alkyl-linked nucleotide can be used to detect and isolate chemical compounds that specifically bind to the nucleotide. For example, an alkyl-  
20 linked nucleotide can be mixed with a combinatorial library. Compounds of the combinatorial library that specifically interact with the alkyl-linked nucleotide can be separated from non-specifically-interacting compounds, for example, by washing with one or more suitable buffers. Subsequently, the specifically-interacting compounds can be identified using art-standard techniques.

25 Alternatively, competitors of compounds which are known to interact with a nucleotide can be identified. For example, an alkyl-linked nucleotide can be mixed with a known interacting compound, such as a protein, to allow for their binding to occur. Then another compound or library of compounds (such as a combinatorial library) can be added to the alkyl-linked nucleotide bound to the known compound. If one or more  
30 different compounds can compete for binding to the nucleotide (and thus, displace the first bound compound), the known compound will be released and the competitor compound can be subsequently identified. Such compounds may be useful biological or pharmacological inhibitors.

5

## EXEMPLIFICATION

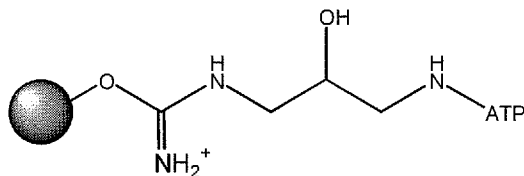
The present invention is further illustrated by the following examples, which are not intended to be limiting in any way. For the purpose of simplicity of illustration, the solid support is generically represented as:



10 wherein, generally a hydroxyl group on the solid support is available to react with a suitable reagent, for example cyanogen bromide which will form a cyanogen bromide-activated solid support. The solid support may be any suitable solid support as described above.

### 15 EXAMPLE 1

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 1:



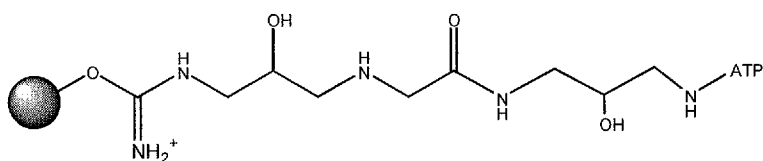
20 The linker arm was attached to the resin by adding 1g of 1,3-diamino-2-propanol to 30mL coupling buffer and combining with 2g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 3 hours at room temperature.

The affinity ligand, ATP, was prepared for attachment to the resin by adding 500  
25 mg ATP, 103.5mg N-hydroxysuccinimide and 172.5mg EDC to 20mL water, and reacting for approximately 2 hours at room temperature.

Finally, the nucleotide affinity media was prepared by adding 111mg 4-dimethylaminopyridine to the activated affinity ligand and combining with the prepared resin and allowing the reaction to continue for approximately 12-18 hours, or overnight,  
30 at room temperature.

## 5 EXAMPLE 2

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 2:



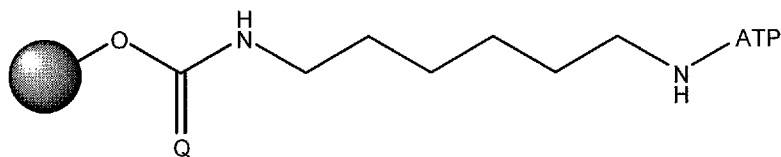
10 The linker was attached to the resin by first adding 1g 1,3-diamino-2-propanol to 30mL coupling buffer and combining with 2g cyanogen bromide-activated SEPHAROSE™. The reaction proceeded for approximately 3 hours. A second reaction of the resin was performed by adding 1.5g iodoacetic acid to 30mL coupling buffer, and adjusting the pH to 9.8 and combining with the resin. This was reacted for a further 1  
15 hour, approximately, at room temperature. In a further reaction with the resin, 500 mg EDC was added to 20mL water and combined with the resin. Reaction proceeded for a further 30 minutes. Subsequently, 1g 1,3-diamino-2-propanol was added to 30 mL coupling buffer and combined with the resin. Reaction continued for another 1 hour, approximately.

20 The affinity ligand was prepared by adding 500 mg ATP, 103.5mg N-hydroxysuccinimide and 172.5mg EDC to 20mL water, and allowing the reaction to proceed for approximately 2 hours.

Finally, 111mg 4-dimethylaminopyridine was added to the prepared affinity ligand and combined with resin. Reaction was allowed to proceed for approximately 12-  
25 18 hours, or overnight at room temperature.

## EXAMPLES 3-8

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples  
30 3-8:



5        wherein Q =  $\text{NH}_2^+$  or O

### EXAMPLE 3

To prepare the resin, 250 mg 1,6-diaminohexane was added to 5mL coupling buffer, combined with 500mg cyanogen bromide-activated SEPHAROSE™ and reacted for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 300 mg ATP, 104mg EDC and 62mg N-hydroxysuccinimide to 7mL water, reacting for 90 minutes at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

15

### EXAMPLE 4

To prepare the resin, 250mg 1,6-diaminohexane was added to 5mL coupling buffer and combined with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction proceeded for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 690mg ATP, 513μL 1-methyl imidazole and 1200mg EDC to 6mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

25

### EXAMPLE 5

To prepare the resin, 750mg 1,6-diaminohexane was added to 7.5mL coupling buffer and combined with 1.425g cyanogen bromide-activated SEPHAROSE™. The reaction proceeded for approximately 2 hours at room temperature.

5           The affinity ligand was prepared by adding 1378mg ATP, 1027μL 1-methyl  
imidazole and 2400mg EDC to 6mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 6

          To prepare the resin, 225mg 1,6-diaminohexane was added to 1.5mL coupling  
buffer and combine with 285mg cyanogen bromide-activated SEPHAROSE™. The  
reaction proceeded for approximately 2 hours at room temperature.

15           The affinity ligand was prepared by adding 550 mg ATP, 410μL 1-methyl  
imidazole and 960mg EDC to 5mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

20

#### EXAMPLE 7

          To prepare the resin, 150 mg 1,6-diaminohexane was added to 3mL coupling  
buffer, the pH was adjusted to pH 8.4, and then combined with 571mg cyanogen bromide  
activated SEPHAROSE™. The reaction proceeded for approximately 2 hours at room  
25   temperature.

          The affinity ligand was prepared by adding 1100mg ATP, 820μL 1-methyl  
imidazole and 1920mg EDC to 10mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and affinity ligand were then combined together and allowed to react  
30   for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 8

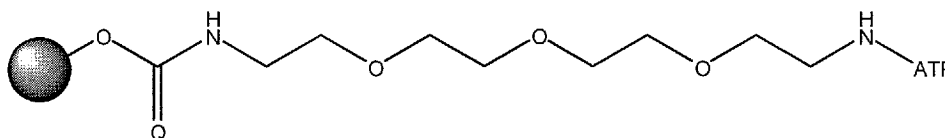
          To prepare the resin, 250mg 1,6-diaminohexane was added to 5mL coupling  
buffer and combines with 2.5mL CDI-activated cross-linked SEPHAROSE™. The  
35   reaction proceeded for approximately 2 hours at room temperature.

5           The affinity ligand was prepared by adding 690mg ATP, 513μL 1-methyl imidazole and 1200mg EDC to 6mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### 10       EXAMPLES 9-28

          The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 9-28:



15       wherein Q = NH<sub>2</sub><sup>+</sup> or O

For all Examples 9-16, the resin was prepared by adding 700 μL 1,11-diamino-3,6,9-trioxaundecane to 10mL coupling buffer and combining with 2g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature. Aliquots of 300 mg of the reacted resin were used for

20       Examples 9-16.

#### EXAMPLE 9

          The affinity ligand was prepared by adding 275mg ATP, 58mg N-hydroxysuccinimide and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand and 300mg of prepared resin (above) were combined and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 10

30       The affinity ligand was prepared by adding 275mg ATP, 41μL 1-methyl imidazole and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

5           The affinity ligand and 300mg of prepared resin (above) were combined and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 11

10           The affinity ligand was prepared by adding 275mg ATP, 290mg N-hydroxysuccinimide and 480mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand and 300mg of prepared resin (above) were combined and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### 15   EXAMPLE 12

          The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

20           The affinity ligand and 300mg of prepared resin (above) were combined and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 13

25           The affinity ligand was prepared by adding 275mg ATP, 58mg N-hydroxysuccinimide and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 5 hours at room temperature.

30           Alternatively, the affinity ligand was prepared by adding 275mg ATP, 58mg N-hydroxysuccinimide and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5     EXAMPLE 14

          The affinity ligand was prepared by adding 275mg ATP, 41μL 1-methyl imidazole and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for  
10    approximately 5 hours at room temperature.

          Alternatively, the affinity ligand was prepared by adding 275mg ATP, 41μL 1-methyl imidazole and 96mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for  
15    approximately 12-18 hours, or overnight at room temperature.

          EXAMPLE 15

          The affinity ligand was prepared by adding 275mg ATP, 290mg N-hydroxysuccinimide and 480mg EDC to 3mL water, and reacting for approximately 2  
20    hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 5 hours at room temperature.

          Alternatively, the affinity ligand was prepared by adding 275mg ATP, 290mg N-hydroxysuccinimide and 480mg EDC to 3mL water, and reacting for approximately 2  
25    hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 12-18 hours, or overnight at room temperature.

          EXAMPLE 16

30        The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 5 hours at room temperature.



5           Alternatively, the affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 3mL water, and reacting for approximately 2 hours at room temperature.

          The affinity ligand was then combined with 300mg resin and allowed to react for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 17

          The resin was prepared by adding 500μL 1,11-diamino-3,6,9-trioxaundecane to 10mL coupling buffer and combining with 2mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at  
15 room temperature.

          The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 8mL water, and reacting for approximately 1 hour at room temperature.

          The affinity ligand was combined with the prepared resin and allowed to react for  
20 approximately 12-18 hours, or overnight at room temperature.

          Alternatively, the affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

          The affinity ligand was combined with the prepared resin and allowed to react for  
25 approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 18

          The resin was prepared by adding 200μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked  
30 SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

5           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature..

#### EXAMPLE 19

10           The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

15           The affinity ligand was prepared by adding 275mg ATP, 205μL 1-methyl imidazole and 480mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 20

20           The resin was prepared by adding 250μL 1,11-diamino-3,6,9-trioxaundecane to 5mL coupling buffer and combining with 2.5mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25           The affinity ligand was prepared by adding 275mg ATP, 82μL 1-methyl imidazole and 192mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

30           Alternatively, the affinity ligand was prepared by adding 250mg ATP, 195μL 1-methyl imidazole and 430mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5     EXAMPLE 21

The resin was prepared by adding 75 $\mu$ L ethanolamine and 75 $\mu$ L 1,11-diamino-3,6,9-trioxaundecane to 3mL coupling buffer and combining with 1.5mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

10       The affinity ligand was prepared by adding 415mg ATP, 125 $\mu$ L 1-methyl imidazole and 290mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

15

EXAMPLE 22

The resin was prepared by adding 135 $\mu$ L ethanolamine and 15 $\mu$ L 1,11-diamino-3,6,9-trioxaundecane to 3mL coupling buffer and combining with 1.5mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 20 hours at room temperature.

The affinity ligand was prepared by adding 415mg ATP, 125 $\mu$ L 1-methyl imidazole and 290mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

25       The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

EXAMPLE 23

30       The resin was prepared by adding 142.5 $\mu$ L ethanolamine and 7.5 $\mu$ L 1,11-diamino-3,6,9-trioxaundecane to 3mL coupling buffer and combining with 1.5mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 415mg ATP, 125 $\mu$ L 1-methyl imidazole and 290mg EDC to 4mL water, and reacting for approximately 1 hour at room temperature.

5           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 24

10           The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked SEPHAROSE™. The reaction was reacted for approximately 2 hours at room temperature.

15           The affinity ligand was prepared by adding 275mg ATP, 82μL 1-methyl imidazole and 192mg EDC to 3mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature..

#### EXAMPLE 25

20           The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25           The affinity ligand was prepared by adding 138mg ATP, 103μL 1-methyl imidazole and 240mg EDC to 3mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### 30   EXAMPLE 26

          The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to 2mL coupling buffer and combining with 1mL CDI-activated cross-linked SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

5           The affinity ligand was prepared by adding 138mg ATP, 41μL 1-methyl  
imidazole and 96mg EDC to 3mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 27

          The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to  
2mL coupling buffer and combining with 1mL CDI-activated cross-linked  
SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at  
15 room temperature.

          The affinity ligand was prepared by adding 275mg ATP, 204μL 1-methyl  
imidazole and 96mg EDC to 3mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
20 for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 28

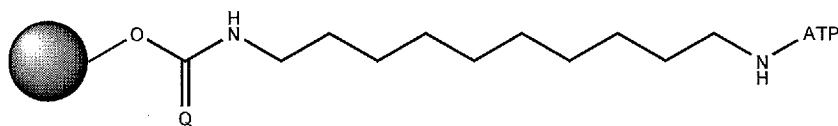
          The resin was prepared by adding 100μL 1,11-diamino-3,6,9-trioxaundecane to  
2mL coupling buffer and combining with 1mL CDI-activated cross-linked  
25 SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at  
room temperature.

          The affinity ligand was prepared by adding 275mg ATP, 41μL 1-methyl  
imidazole and 480mg EDC to 3mL water, and reacting for approximately 1 hour at room  
temperature.

30           The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

5   EXAMPLES 29-36

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 29-36:



10   wherein Q = NH<sub>2</sub><sup>+</sup> or O

EXAMPLE 29

The resin was prepared by adding 250 mg 1,10-diaminodecane and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 571 mg cyanogen bromide-activated  
15   SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 550 mg ATP, 410 μL 1-methyl imidazole and 960 mg EDC to 6 mL water, and reacting for approximately 1 hour at room temperature.

20   The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

EXAMPLE 30

The resin was prepared by adding 125mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated  
25   SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 482 mg ATP, 359 μL 1-methyl imidazole and 840 mg EDC to 5.25 mL water, and reacting for approximately 1 hour at  
30   room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5    EXAMPLE 31

The resin was prepared by adding 250mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

10    The affinity ligand was prepared by adding 482mg ATP, 359μL 1-methyl imidazole and 840mg EDC to 5.25mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

15

EXAMPLE 32

The resin was prepared by adding 219mg 1,10-diaminodecane and 0.875mL 1,4-dioxane to 3.5mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2  
20 hours at room temperature.

The affinity ligand was prepared by adding 482mg ATP, 359μL 1-methyl imidazole and 840mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react  
25 for approximately 12-18 hours, or overnight at room temperature.

EXAMPLE 33

The resin was prepared by adding 219mg 1,10-diaminodecane and 0.875mL 1,4-dioxane to 3.5mL coupling buffer and combining with 500mg cyanogen bromide-  
30 activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

5           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 34

10           The resin was prepared by adding 125mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

15           The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 35

20           The resin was prepared by adding 250 mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25           The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### 30   EXAMPLE 36

          The resin was prepared by adding 125mg 1,10-diaminodecane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.



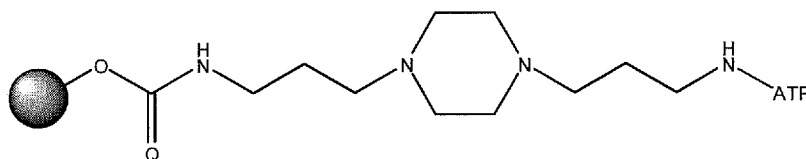
5           The affinity ligand was prepared by adding 482mg ATP, 359μL 1-methyl imidazole and 840mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 37

          The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 37:



15

wherein Q = NH<sub>2</sub><sup>+</sup> or O

          The resin was prepared by adding 125μL 1,4-bis(3-aminopropyl)piperazine and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

20

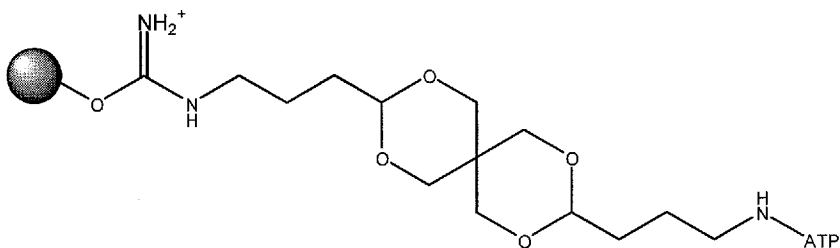
          The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

25

#### EXAMPLE 38

          The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 38:



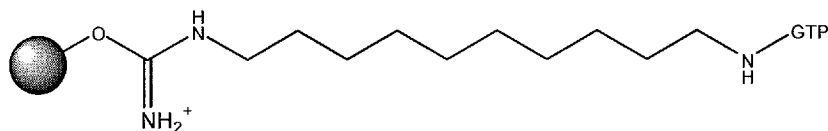
The resin was prepared by adding 125mg 2,4,8,10-tetraoxaspiro[5.5]undecane-3,9-dipropanamine and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 241mg ATP, 180μL 1-methyl imidazole and 40mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 39

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 39:



The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

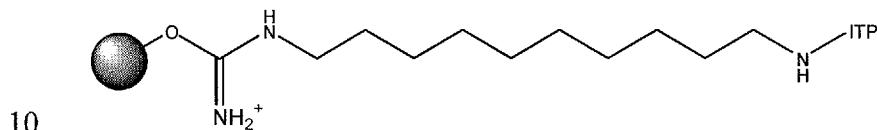
The affinity ligand was prepared by adding 39mg GTP, 31μL 1-methyl imidazole and 72mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5

## EXAMPLE 40

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 40:



The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

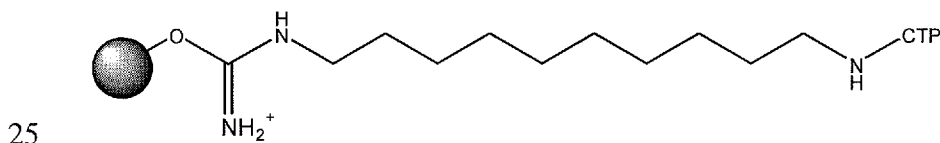
15 The affinity ligand was prepared by adding 43mg ITP, 31μL 1-methyl imidazole and 72mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

20

## EXAMPLE 41

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 41:



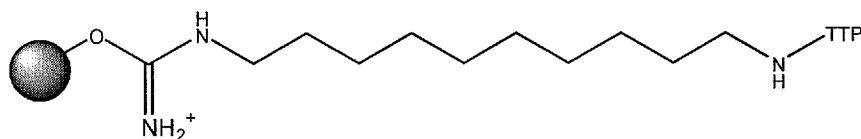
The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

30 The affinity ligand was prepared by adding 40mg CTP, 31μm 1-methyl imidazole and 72mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

5           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 42

10           The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 42:



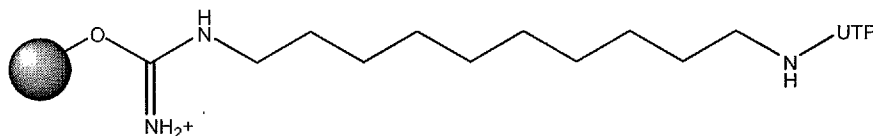
15           The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 36 mg TTP, 31 $\mu$ L 1-methyl imidazole and 72 mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

20           The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 43

25           The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 43:



30           The resin was prepared by adding 62.5mg 1,10-diaminodecane and 0.25mL 1,4-dioxane to 1mL coupling buffer and combining with 140mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

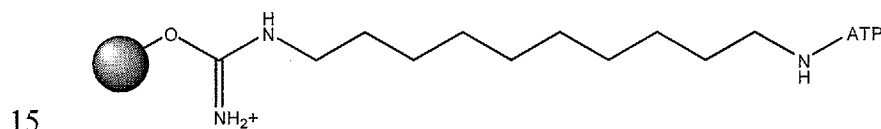
5           The affinity ligand was prepared by adding 37mg UTP, 31μL 1-methyl imidazole and 72 mg EDC to 1mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature. React overnight.

10

#### EXAMPLES 44-48

          The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 44-48:



#### EXAMPLE 44

          The resin was prepared by adding 187.5mg 1,10-diaminodecane, 62.5μL ethanolamine and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature..

          The affinity ligand was prepared by adding 480mg ATP, 360μL 1-methyl imidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 45

          The resin was prepared by adding 125mg 1,10-diaminodecane, 125μL ethanolamine and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

5           The affinity ligand was prepared by adding 480mg ATP, 360μL 1-methyl  
imidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

10

#### EXAMPLE 46

          The resin was prepared by adding 62.5mg 1,10-diaminodecane, 187.5μL  
ethanolamine and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g  
cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for  
15 approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 480mg ATP, 360μL 1-methyl  
imidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room  
temperature.

          The resin and affinity ligand were then combined together and allowed to react  
20 for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 47

          The resin was prepared by adding 25mg 1,10-diaminodecane, 225μL  
ethanolamine and 1mL, 1,4-dioxane to 4mL coupling buffer and combining with 1g  
25 cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for  
approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 480 mg ATP, 360 μL 1-methyl  
imidazole and 840 mg EDC to 5 mL water, and reacting for approximately 1 hour at  
room temperature.

30           The resin and affinity ligand were then combined together and allowed to react  
for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 48

          The resin was prepared by adding 83 mg 1,10-diaminodecane, 167 μL  
35 ethanolamine and 1 mL 1,4-dioxane to 4 mL coupling buffer and combining with 1 g

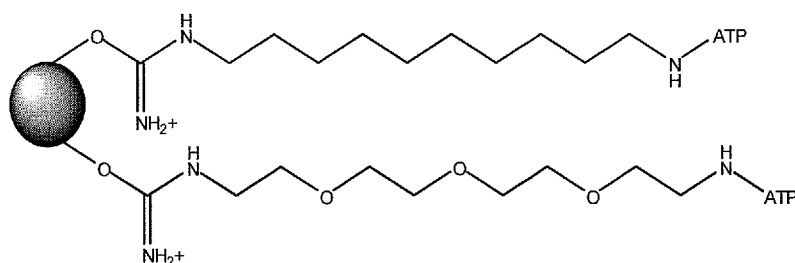
5 cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 480 mg ATP, 360  $\mu$ L 1-methyl imidazole and 840 mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

10 The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLES 49-50

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linkers and reaction conditions described in  
15 Examples 49-50:



#### EXAMPLE 49

20 The resin was prepared by adding 125mg 1,10-diaminodecane, 125 $\mu$ L 1,11-diamino-3,6,9-trioxaundecane and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 480mg ATP, 360 $\mu$ L 1-methyl imidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room  
25 temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

5    EXAMPLE 50

The resin was prepared by adding 62.5mg 1,10-diaminodecane, 187.5μL 1,11-diamino-3,6,9-trioxaundecane and 1mL 1,4-dioxane to 4mL coupling buffer and combining with 1g cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

10    The affinity ligand was prepared by adding 480mg ATP, 360μL 1-methylimidazole and 840mg EDC to 5mL water, and reacting for approximately 1 hour at room temperature.

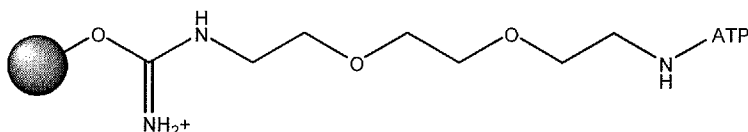
The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

15

EXAMPLE 51

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example

20    51:



25    The resin was prepared by adding 125μL 1,8-diamino-3,6-dioxaoctane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

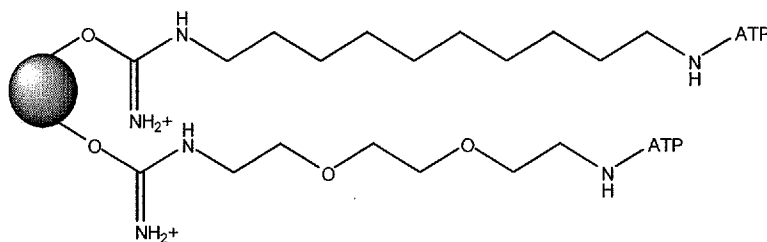
30    The affinity ligand was prepared by adding 240mg ATP, 180μL 1-methylimidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.



5   EXAMPLES 52-53

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linkers and reaction conditions described in Examples 52-53:



10

EXAMPLE 52

The resin was prepared by adding 62.5mg 1,10-diaminodecane, 62.5μL 1,8-diamino-3,6-dioxaoctane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

15

The affinity ligand was prepared by adding 240mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

20

EXAMPLE 53

The resin was prepared by adding 31.5mg 1,10-diaminodecane, 93.75μL 1,8-diamino-3,6-dioxaoctane and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

25

The affinity ligand was prepared by adding 240mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

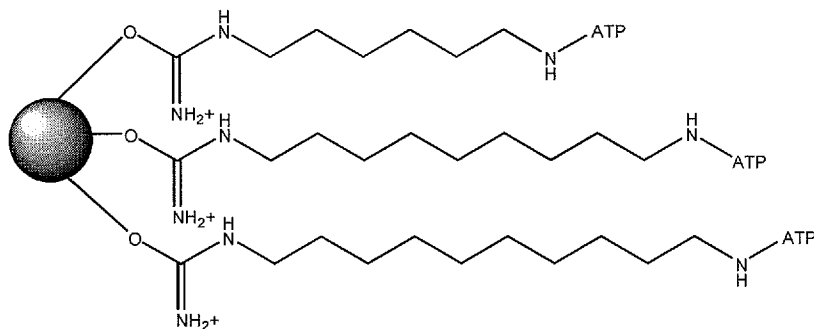
The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

30

5

## EXAMPLES 54-56

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linkers and reaction conditions described in Examples 54-56:



10

For Examples 54-56, a mixture of 100mg of each linker arm: 1,10-diaminodecane; 1,9-diaminononane; and 1,6-diaminohexane, was prepared for use in the preparation of the nucleotide affinity media described.

## 15 EXAMPLE 54

The resin was prepared by adding 125mg of the mixture of linker arms (above), and 0.5mL 1,4-dioxane to 2mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

20 The affinity ligand was prepared by adding 240mg ATP, 180  $\mu$ L 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

25

## EXAMPLE 55

The resin was prepared by adding 62.5 mg of the mixture of linker arms (above), 62.5  $\mu$ L ethanolamine and 0.5mL 1,4-dioxane to 2 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

30

5 The affinity ligand was prepared by adding 240 mg ATP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### 10 EXAMPLE 56

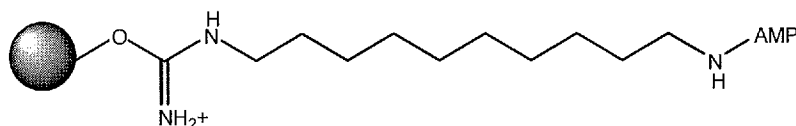
The resin was prepared by adding 31.25 mg of the mixture of linker arms (above), 93.75  $\mu$ L ethanolamine and 0.5 mL 1,4-dioxane to 2 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

15 The affinity ligand was prepared by adding 240 mg ATP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

20 The resin and affinity ligand were then combined together and allowed to react for approximately 12-18 hours, or overnight at room temperature.

#### EXAMPLE 57

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 25 57:



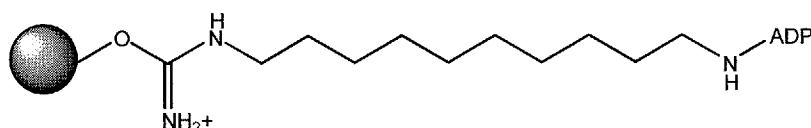
30 The resin was prepared by adding 125 mg 1,10-diaminodecane and 0.5 mL 1,4-dioxane to 2 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 160mg AMP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

5           The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 58

10           The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 58:



15           The resin was prepared by adding 125 mg 1,10-diaminodecane and 0.5 mL 1,4-dioxane to 2 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

          The affinity ligand was prepared by adding 188 mg ADP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

20           The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

General procedure for SEPHACRYL™ and TOYOPEARL® resins:

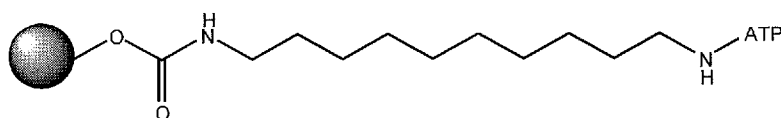
25           SEPHACRYL™ and TOYOPEARL® resins are generally stored in 20% ethanol. After washing 2.5 mL of the resin with 2 volumes of water, 1 volume of 30% acetone, 1 volume of 70% acetone and 5 volumes of dry acetone the resin is transferred into 2.5 mL dry acetone. 200 mg 1,1'-carbonyl diimidazole is added and the resin is allowed to react for approximately 1 hour at room temperature. After approximately 1 hour the resin is washed with 5 volumes of dry acetone, 1 volume of 70% acetone, 1 volume of 30% acetone and 2 volumes of water. 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of a suitable coupling buffer and combined with the resin. The resin is allowed to react overnight at 4°C. The resin is then washed with three volumes of 1 M NaCl and two volumes of water. To end-cap any remaining reactive sites, 5 mL of a 1 M

30

5 ethanolamine solution (pH = 8.9) is added to the resin and the resin is allowed to react at room temperature for approximately 1 hour. The resin is subsequently washed with three volumes of 1 M NaCl and two volumes of water. 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC are added to 10 mL water, and after reacting for approximately 1 hour, are combined with the resin. The resin is allowed to react  
10 overnight. Finally the resin is washed with three volumes of 1 M NaCl and two volumes of water and transferred into a storage buffer.

#### EXAMPLES 59-64

The following general structure represents the affinity ligand bound to a solid  
15 support when synthesized using the linker and reaction conditions described in Examples 59-64:



#### EXAMPLE 59

20 The resin is prepared by combining 2.5 mL of HR S-300 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any  
25 remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

The affinity ligand is prepared by adding 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and  
30 affinity ligand are reacted for approximately 12-18 hours, or overnight.

5   EXAMPLE 60

          The resin is prepared by combining 2.5 mL of HR S-300 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined  
10   with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

          The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and  
15   affinity ligand are reacted for approximately 12-18 hours, or overnight.

EXAMPLE 61

          The resin is prepared by combining 2.5 mL of HR S-300 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed  
20   to proceed for approximately 1 hour. Next, 62.5 mg 1,10-diaminodecane, 187.5 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

          The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl  
25   imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

EXAMPLE 62

30       The resin is prepared by combining 2.5 mL of HR S-400 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any

5 remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

The affinity ligand is prepared by adding 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and  
10 affinity ligand are reacted for approximately 12-18 hours, or overnight.

#### EXAMPLE 63

The resin is prepared by combining 2.5mL of HR S-400 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed  
15 to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combine with resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720  $\mu$ L 1-methyl  
20 imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

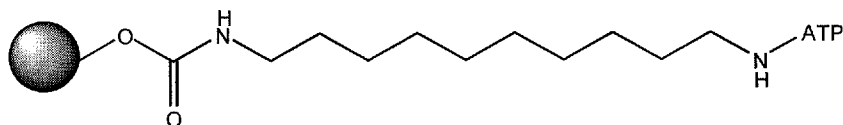
#### EXAMPLE 64

25 The resin is prepared by combining 2.5 mL of HR S-400 SEPHACRYL™ resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 62.5mg 1,10-diaminodecane, 187.5 $\mu$ L ethanolamine and 1mL 1,4-dioxane are added to 4mL of coupling buffer and combine with resin. The reaction is allowed to proceed for approximately 12-18 hours, or  
30 overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and  
35 affinity ligand are reacted for approximately 12-18 hours, or overnight.

5   EXAMPLES 65-70

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 65-70:



10

EXAMPLE 65

The resin is prepared by combining 2.5 mL of HW-65F TOYOPEARL® resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next 250 mg 1,10-diaminodecane and 1mL 1,4-dioxane are added to 4mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

EXAMPLE 66

The resin is prepared by combining 2.5 mL of HW-65F TOYOPEARL® resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for



- 5 approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

#### EXAMPLE 67

- The resin is prepared by combining 2.5 mL of HW-65S TOYOPEARL® resin  
10 with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution  
15 to the resin and reacting for approximately 1 hour.

- The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl  
imidazole and 1680 mg EDC to 10mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.  
20

#### EXAMPLE 68

- The resin is prepared by combining 2.5 mL of HW-65S TOYOPEARL® resin  
with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed  
25 to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

- The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl  
30 imidazole and 1680 mg EDC to 10mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

5     **EXAMPLE 69**

          The resin is prepared by combining 2.5 mL of HW-75F TOYOPEARL® resin with 2.5 mL dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 250 mg 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction  
10    is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. Any remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

          The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for  
15    approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

**EXAMPLE 70**

          The resin is prepared by combining 2.5mL of HW-75F TOYOPEARL® resin  
20    with 2.5mL dry acetone and 200mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

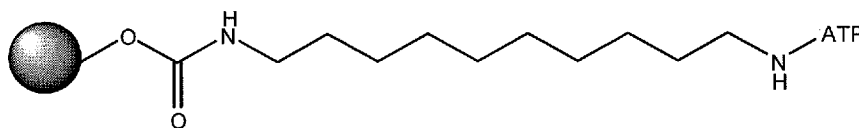
25        The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

30        The procedure for the AF-Tresyl-650M TOYOPEARL® resin is different than for the other TOYOPEARL® resins, since this resin comes with its hydroxyl functionalities pre-activated with tresyl chloride (an organic sulfonyl chloride), similar to cyanogen bromide-activated SEPHAROSE™ or 1,1'-carbonyl diimidazole-activated SEPHAROSE™.

35

5   EXAMPLES 71-72

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 71-72:



10

EXAMPLE 71

The resin is prepared by allowing 0.5 g of AF-Tresyl-650M TOYOPEARL® resin to swell in 15 mL 1mM HCl for approximately 10 minutes. The resin is then washed with 2 volumes of 1mM HCl and 1 volume of water. Next, 250 mg 1,10-  
15   diaminodecane and 1 mL 1,4-dioxane are added to 4 mL of coupling buffer (0.2 M sodium phosphate buffer, pH = 7.45) and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4° C. Any remaining reactive sites are end-capped by adding 5 mL of a 1 M ethanolamine solution to the resin and reacting for approximately 1 hour.

20       The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water. The reaction is allowed to proceed for approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

25   EXAMPLE 72

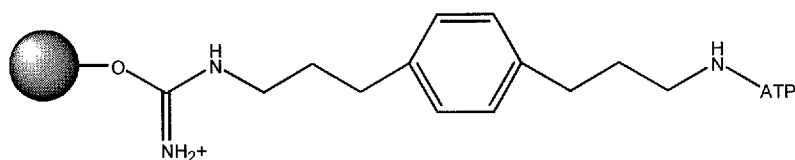
The resin is prepared by allowing 0.5 g of AF-Tresyl-650M TOYOPEARL® resin to swell in 15 mL HCl for approximately 10 minutes. The resin is then washed with 2 volumes of 1 mM HCl and 1 volume of water. Next, 125 mg 1,10-diaminodecane, 125 µL ethanolamine and 1mL 1,4-dioxane are added to 4mL of coupling buffer (0.2M  
30   sodium phosphate buffer, pH = 7.45) and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C.

The affinity ligand is prepared by adding 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10mL water. The reaction is allowed to proceed for

- 5 approximately 1 hour before combining with the prepared resin. The combined resin and affinity ligand are reacted for approximately 12-18 hours, or overnight.

#### EXAMPLE 73

- 10 The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 73:



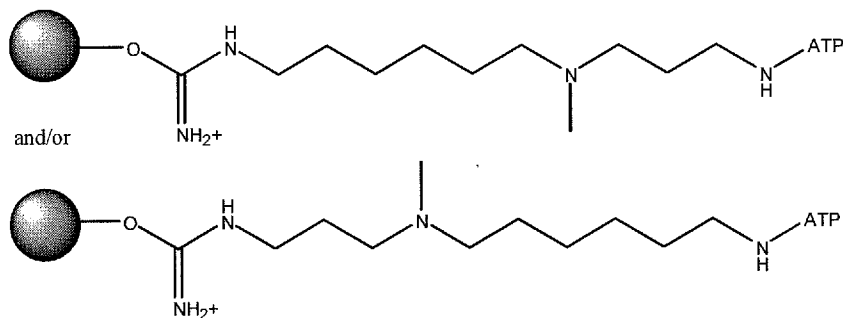
- 15 The resin was prepared by adding 190 mg 3-[4-(3-aminopropyl)-phenyl]-propylamine and 1.5 mL 1,4-dioxane to 3 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 5 hours at room temperature.

The affinity ligand was prepared by adding 480 mg ATP, 360  $\mu$ L 1-methylimidazole and 840 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

- 20 The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 74

- 25 The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 74:



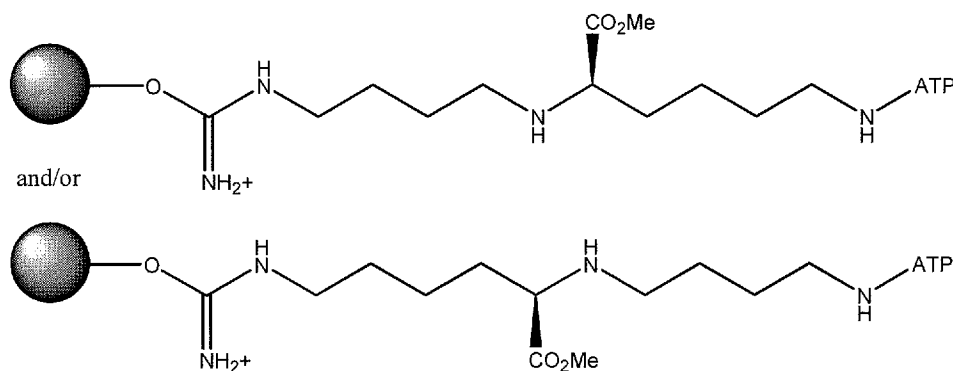
5        The resin was prepared by adding 190 mg N-(3-aminopropyl)-N-methyl-hexane-1,6-diamine and 0.75 mL 1,4-dioxane to 3 mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 5 hours at room temperature.

      The affinity ligand was prepared by adding 480 mg ATP, 360μL 1-methyl  
10 imidazole and 840 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

      The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

## 15    EXAMPLE 75

      The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 75:



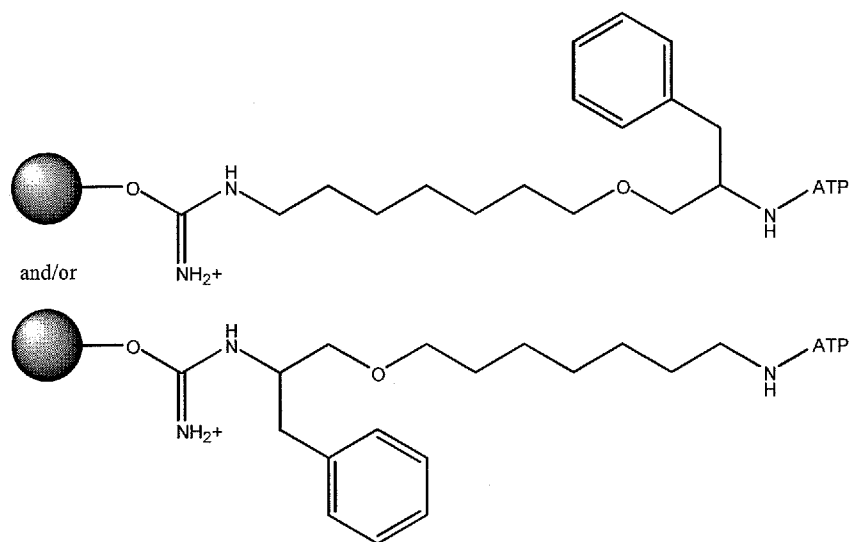
20        The resin was prepared by adding 350 mg 6-amino-2-(4-amino-butylamino)-hexanoic acid methyl ester and 1.4 mL 1,4-dioxane to 5.6 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 5 hours at room temperature.

      The affinity ligand was prepared by adding 480 mg ATP, 360 μL 1-methyl  
25 imidazole and 840 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

      The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

## 5 EXAMPLE 76

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 76:



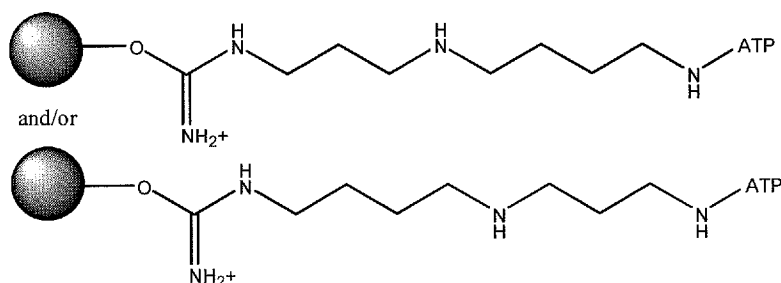
10 The resin was prepared by adding 260 mg 7-(2-amino-3-phenyl-propoxy)-heptylamine and 1.0 mL 1,4-dioxane to 4 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 5 hours at room temperature.

The affinity ligand was prepared by adding 480 mg ATP, 360  $\mu$ L 1-methyl  
15 imidazole and 840 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

## 20 EXAMPLE 77

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 77:



The resin was prepared by adding 105 mg spermidine and 0.5 mL 1,4-dioxane to 2.5 mL coupling buffer and combining with 500 mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

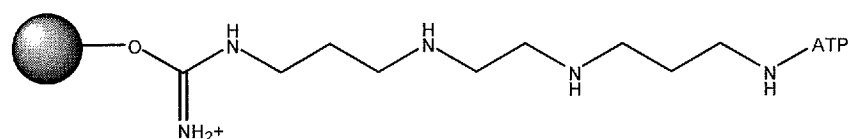
10 The affinity ligand was prepared by adding 240 mg ATP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 78

The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example

78:



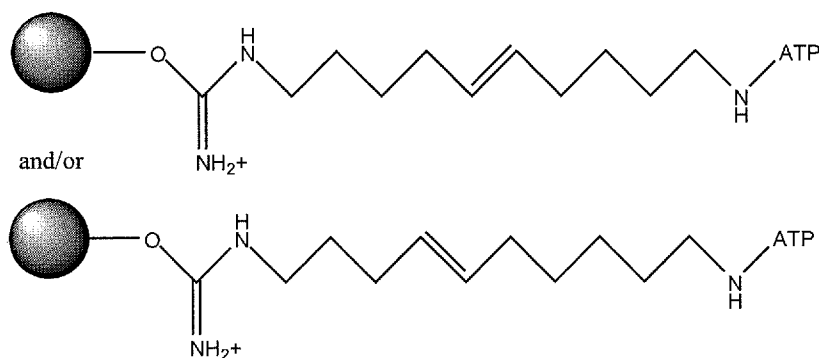
25 The resin was prepared by adding 127 mg bis(3-aminopropyl)-ethylene diamine and 0.5 mL 1,4-dioxane to 2.5 mL coupling buffer and combining with 500mg cyanogen bromide-activated SEPHAROSE™. The reaction was allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand was prepared by adding 240 mg ATP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

- 5           The resin and the affinity ligand were combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 79

- 10           The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Example 79:



- 15           1, 10 Diiodo-dec-5-ene is prepared as follows: 9-Borabicyclo[3.3.1]nonane (1 equiv.) is added to anhydrous THF at 0° C followed by followed by 1, 5, 9-decatriene (4 equiv.). The reaction is warmed to ambient temperature and stirred for 1.5 h. The resulting solution is cooled to -20° and sodium methoxide (2 equiv.) is added followed by iodine (2 equiv.). The reaction mixture is stirred at -20°C for 1.5 h, warmed to room temperature and stirred for an additional 1 h. The crude product is purified via silica gel chromatography. The appropriate fractions are pooled and concentrated in vacuo to afford 1, 10-Diiodo-dec-5-ene.

- 20           The 1, 10-Diiodo-dec-5-ene (1 equiv.) is dissolved in anhydrous acetone at ambient temperature followed by the addition of potassium phthalimide (4. equiv.). The reaction mixture is stirred until completion, washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The crude product is purified via silica gel chromatography to afford 1, 10-dipthalimido-dec-5-ene.

25           The 1, 10-dipthalimido-dec-5-ene (1 equiv.) and hydrazine (2 equiv.) are stirred in ethanol at reflux. The reaction mixture is cooled to room temperature and the resulting

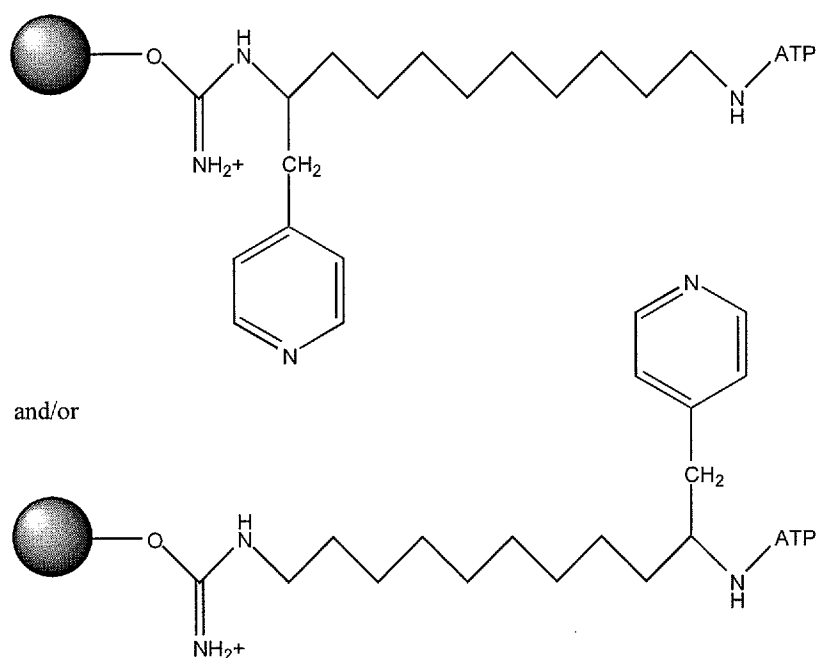


5 solid is filtered off. The filtrate is purified via silica gel chromatography to yield 1, 10  
diamino-dec-5-ene.

200 mg of the linker arm and 1.5ml 1,4-dioxane are added to 3ml coupling buffer  
and combined with 500 mg cyanogen bromide-activated Sepharose. The reaction is  
allowed to proceed for about five hours. 480mg ATP, 360ml 1-methyl imidazole and  
10 840mg EDC are added to 2.5ml water, allowed to react for one hour, and then combined  
the resin. The reaction is allowed to proceed overnight.

#### EXAMPLE 80

The following general structure represents the affinity ligand bound to a solid  
15 support when synthesized using the linker and reaction conditions described in Example  
80:



Magnesium (1.2 equiv.) is stirred in anhydrous ether under a nitrogen atmosphere  
20 and a catalytic amount of iodine is added. The mixture is heated to reflux and 4-  
bromomethylpyridine (1 equiv.) is added. The reaction is stirred until formation of the  
Grignard reagent is complete and 9-phthalimidononanal (1.5 equiv.) is added. The  
reaction mixture is stirred until completion, quenched with ammonium chloride, extracted

5 with brine and concentrated in vacuo. The crude product is purified by silica gel chromatography to give 1-hydroxy-1-(4-pyridinyl)-10-phthalimidodecane.

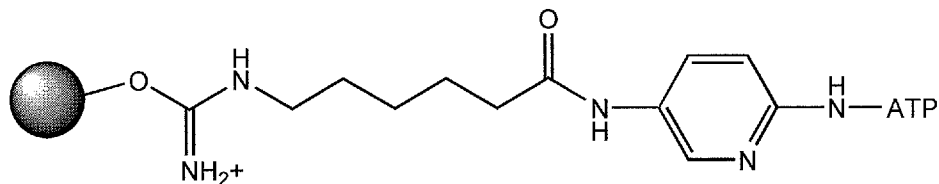
1-Hydroxy-1-(4-pyridinyl)-10-phthalimidodecane (1 equiv.) is dissolved in THF with stirring. Phthalimide (1.5 equiv.) and triphenylphosphine (2.1 equiv.) are added to the solution and the resulting mixture is cooled to 0° C. Diisopropyl azodicarboxylate  
10 (2.0 equiv.) is added dropwise to the above solution and the reaction is stirred until completion. The resulting solid is filtered off and the filtrate is concentrated in vacuo. The crude product is purified by silica gel chromatography to yield 1, 10-diphthalimido-1-pyridinyldecane.

1, 10-diphthalimido-1-pyridinyldecane (1 equiv.) and hydrazine (2 equiv.) are  
15 stirred in ethanol at reflux. The reaction mixture is cooled to room temperature and the resulting solid is filtered off. The filtrate is purified by silica gel chromatography to yield 1, 10-diamino-1-pyridinyldecane.

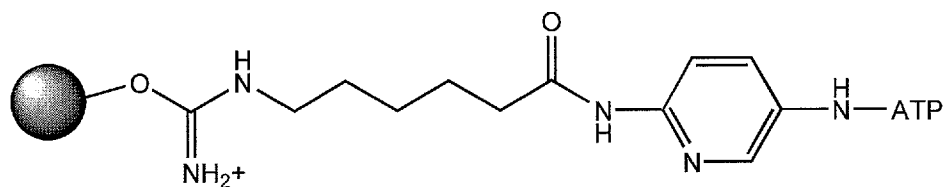
250mg of linker arm and 1.5ml 1, 4-dioxane is added to 3ml coupling buffer and combined with 500mg cyanogen bromide-activated Sepharose. The reaction is allowed  
20 to proceed for 5 hours. 480mg ATP, 360ml 1-methyl-imidazole and 840mg EDC are added to 2.5ml water, reacted for about one hour, and combined with the resin. The reaction is allowed to proceed overnight.

## 25 EXAMPLES 81-83

The following general structures represent the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 81-83:



5



CH-activated SEPHAROSE™ 4B (Sigma) is used for these examples.

#### EXAMPLE 81

10        50 mg of 2,5-diaminopyridine and 0.2 mL 1,4-dioxane are added to 0.8 mL coupling buffer and combined with 200 mg CH-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

15        The affinity ligand is prepared by combining 100 mg ATP, 74  $\mu$ L 1-methyl imidazole and 172 mg EDC to 1.5mL water, and reacting for approximately 1 hour at room temperature.

      The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 82

20        40 mg of 2,5-diaminopyridine, 10 $\mu$ L ethanolamine and 0.2 mL 1,4-dioxane are added to 0.8 mL coupling buffer and combined with 200 mg CH-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

25        The affinity ligand is prepared by combining 100 mg ATP, 74  $\mu$ L 1-methyl imidazole and 172 mg EDC to 1.5 mL water, and reacting for approximately 1 hour at room temperature.

#### EXAMPLE 83

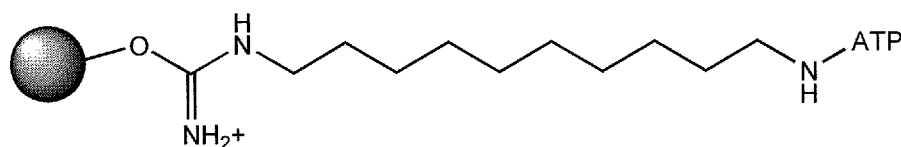
30        30 mg of 2,5-diaminopyridine, 20 $\mu$ L ethanolamine, and 0.2 mL 1,4-dioxane are added to 0.8 mL coupling buffer and combined with 200 mg CH-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

5           The affinity ligand is prepared by combining 100mg ATP, 74 $\mu$ L 1-methyl imidazole and 172 mg EDC to 1.5mL water, and reacting for approximately 1 hour at room temperature.

          The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### 10       EXAMPLES 84-89

          The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 84-89:



Cyanogen bromide-activated SEPHAROSE™ 4B (Sigma) is used for these examples.

#### EXAMPLE 84

112.5 mg of 1,10-diaminodecane, 12.5 mg glycine, and 0.5 mL 1,4-dioxane are added to 2 mL coupling buffer and combined with 0.5 mg cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

25       The affinity ligand is prepared by combining 240 mg ATP, 180  $\mu$ L 1-methyl imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at room temperature.

          The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 85

30       93.75 mg of 1,10-diaminodecane, 31.25 mg glycine, and 0.5 mL 1,4-dioxane are added to 2 mL coupling buffer and combined with 0.5 mg cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

5           The affinity ligand is prepared by combining 240 mg ATP, 180  $\mu$ L 1-methyl  
imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and the affinity ligand are combined and allowed to react for  
approximately 12-18 hours, or overnight, at room temperature.

10

#### EXAMPLE 86

          62.5 mg of 1,10-diaminodecane, 62.5 mg glycine, and 0.5 mL 1,4-dioxane are  
added to 2 mL coupling buffer and combined with 0.5 mg cyanogen bromide-activated  
SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room  
15   temperature.

          The affinity ligand is prepared by combining 240 mg ATP, 180  $\mu$ L 1-methyl  
imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at  
room temperature.

          The resin and the affinity ligand are combined and allowed to react for  
20   approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 87

          112.5 mg of 1,10-diaminodecane, 12.5 mg tris(hydroxymethyl)aminomethane,  
and 0.5 mL 1,4-dioxane are added to 2 mL coupling buffer and combined with 0.5 mg  
25   cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for  
approximately 2 hours at room temperature.

          The affinity ligand is prepared by combining 240 mg ATP, 180  $\mu$ L 1-methyl  
imidazole and 420 mg EDC to 2.5 mL water, and reacting for approximately 1 hour at  
room temperature.

30           The resin and the affinity ligand are combined and allowed to react for  
approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 88

          93.75 mg of 1,10-diaminodecane, 31.25 mg tris(hydroxymethyl)aminomethane,  
35   and 0.5 mL 1,4-dioxane are added to 2 mL coupling buffer and combined with 0.5 mg

5 cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand is prepared by combining 240mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at room temperature.

10 The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 89

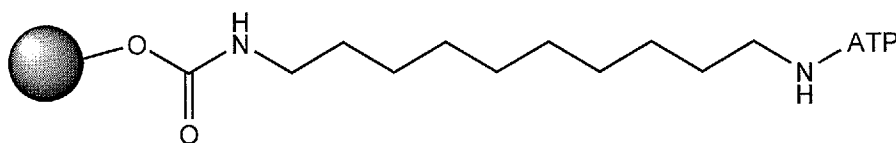
62.25mg of 1,10-diaminodecane, 62.25mg tris(hydroxymethyl)aminomethane,  
15 and 0.5mL 1,4-dioxane are added to mL coupling buffer and combined with 0.5 mg cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature.

The affinity ligand is prepared by combining 240mg ATP, 180μL 1-methyl imidazole and 420mg EDC to 2.5mL water, and reacting for approximately 1 hour at  
20 room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLES 90-96

25 The following general structure represents the affinity ligand bound to a solid support when synthesized using the linker and reaction conditions described in Examples 90-96:



Trisacryl resin GF 2000 M (BioSeptra) or Ultrogel AcA 22 (BioSeptra) resin is  
30 used for these examples. The general procedure for these resins is the same as that described for the TOYOPEARL® and SEPHACRYL™ resins as described above.

5    EXAMPLE 90:

2.5 ml Trisacryl GF 2000 M resin was combined with 2.5 ml dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 250 mg of 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to  
10    proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1 M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at  
15    room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

EXAMPLE 91

20        2.5 ml Trisacryl GF 2000 M resin was combined with 2.5 ml dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 165 mg of 1,10-diaminodecane, 85 µL ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL  
25    of a 1 M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720 µL 1-methyl imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at room temperature.

30        The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

EXAMPLE 92

2.5 ml Trisacryl GF 2000 M resin was combined with 2.5 ml dry acetone and 200  
35    mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1

5 hour at room temperature. 82 mg of 1,10-diaminodecane, 168  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

10 The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

15

#### EXAMPLE 93

2.5 ml Ultrogel AcA 22 resin was combined with 2.5 ml dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 250 mg of 1,10-diaminodecane and 1 mL 1,4-dioxane are added to 20 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1 M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl 25 imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at room temperature.

The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### 30 EXAMPLE 94

2.5 ml Ultrogel AcA 22 resin was combined with 2.5 ml dry acetone and 200 mg 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 165 mg of 1,10-diaminodecane, 85  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is 35 allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1M



5 ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10mL water, and reacting for approximately 1 hour at room temperature.

10 The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 95

2.5 ml Ultrogel AcA 22 resin was combined with 2.5 ml dry acetone and 200 mg  
15 1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour at room temperature. 82 mg of 1,10-diaminodecane, 168  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1M ethanolamine solution are then added to the resin and allowed to react at room  
20 temperature for approximately 1 hour.

The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10 mL water, and reacting for approximately 1 hour at room temperature.

25 The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

#### EXAMPLE 96

2.5 ml Ultrogel AcA 22 resin was combined with 2.5 ml dry acetone and 200 mg  
1,1'-carbonyl diimidazole. The reaction is allowed to proceed for approximately 1 hour  
30 at room temperature. 25 mg of 1,10-diaminodecane, 225  $\mu$ L ethanolamine and 1 mL 1,4-dioxane are added to 4 mL coupling buffer and combined with the resin. The reaction is allowed to proceed for approximately 12-18 hours, or overnight, at 4°C. 5 mL of a 1 M ethanolamine solution are then added to the resin and allowed to react at room temperature for approximately 1 hour.

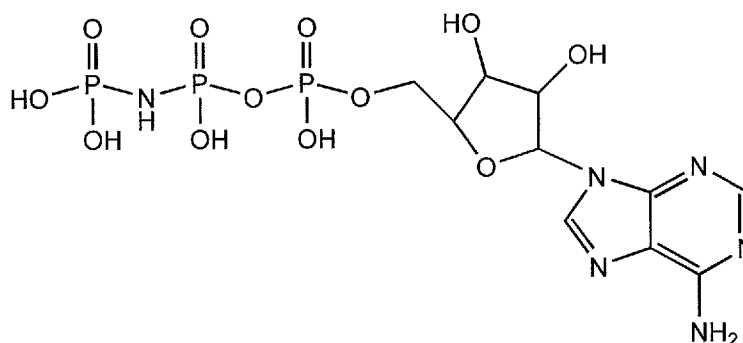
5           The affinity ligand is prepared by combining 964 mg ATP, 720  $\mu$ L 1-methyl imidazole and 1680 mg EDC to 10mL water, and reacting for approximately 1 hour at room temperature.

          The resin and the affinity ligand are combined and allowed to react for approximately 12-18 hours, or overnight, at room temperature.

10

#### EXAMPLE 97

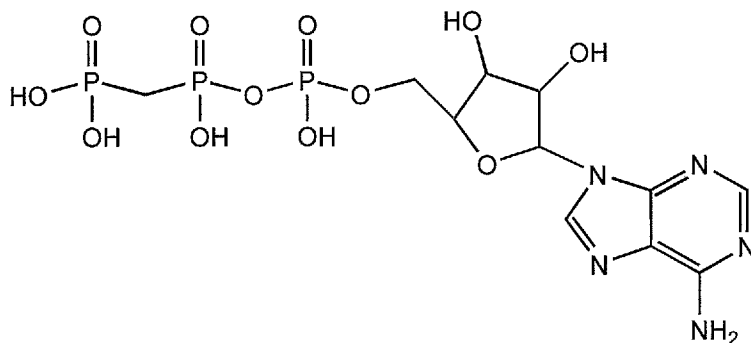
          The following structure represents an ATP analog out of which an alkyl linked nucleotide was made using the conditions described in Example 97:



15           13.5 mg of 1,10-diaminodecane, 13.5  $\mu$ L ethanolamine and 105  $\mu$ L 1,4-dioxane are added to 419  $\mu$ L coupling buffer and combined with 105 mg cyanogen-bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature. 29 mg adenylyl-imido-diphosphate (Calbiochem®, San Diego California), 22.5  $\mu$ L 1-methyl imidazole and 52.5 mg EDC are added to 625  $\mu$ L water, and the reaction  
20 is allowed to proceed for one hour. The resin is combined with the ligand, and the reaction is allowed to proceed overnight.

#### EXAMPLE 98

          The following structure represents an ATP analog out of which an alkyl linked  
25 nucleotide was made using the conditions described in Example 98:



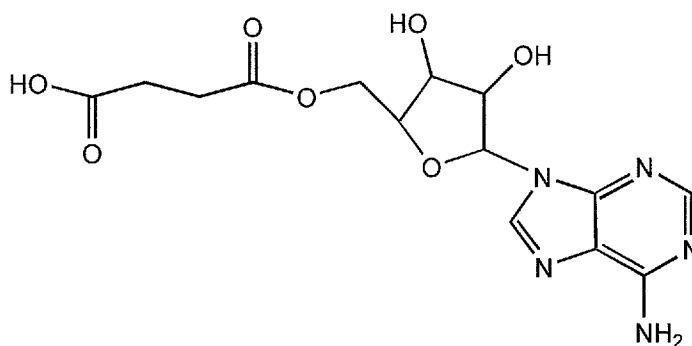
5

13.5 mg of 1,10-diaminodecane, 13.5  $\mu$ l ethanolamine and 105  $\mu$ l 1,4-dioxane are added to 419  $\mu$ l coupling buffer and combined with 105 mg cyanogen-bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature. 30 mg  $\beta,\gamma$ -methylene-ATP (Calbiochem®, San Diego California), 22.5  $\mu$ l 1-methyl imidazole and 52.5 mg EDC are added to 625  $\mu$ l water, and the reaction is allowed to proceed for one hour. The resin is combined with the ligand, and the reaction is allowed to proceed overnight.

#### EXAMPLE 99

15 The following structure represents an ATP analog out of which an alkyl linked nucleotide was made using the conditions described in Example 99:

:



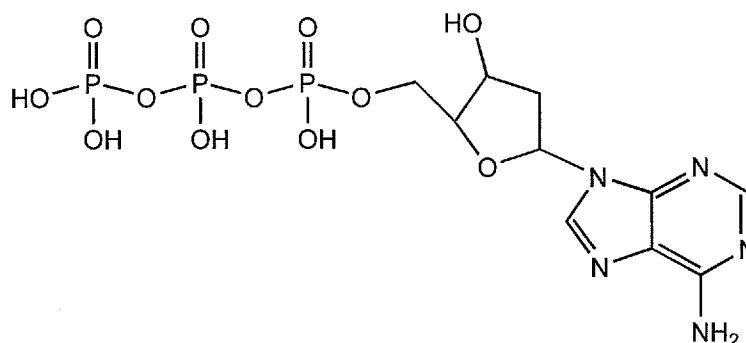
16 mg of 1,10-diaminodecane, 16  $\mu$ l ethanolamine and 125  $\mu$ l 1,4-dioxane are added to 500  $\mu$ l coupling buffer and combined with 125 mg cyanogen bromide-activated SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room temperature. 20 mg adenosine-5'-succinate (Sigma, St. Louis, MO), 22.5  $\mu$ l 1-methyl imidazole and 52.5 mg EDC are added to 625  $\mu$ l water, and the reaction is allowed to

5 proceed for one hour. The resin is combined with the ligand, and the reaction is allowed to proceed overnight.

#### EXAMPLE 100

The following structure represents an ATP analog out of which an alkyl linked  
10 nucleotide was made using the conditions described in Example 100:

:



16 mg of 1,10-diaminodecane, 16  $\mu$ l ethanolamine and 125  $\mu$ l 1,4-dioxane are  
added to 500  $\mu$ l coupling buffer and combined with 125 mg cyanogen-bromide-activated  
15 SEPHAROSE™. The reaction is allowed to proceed for approximately 2 hours at room  
temperature. 29 mg 2-deoxy-ATP (Sigma, St. Louis, MO), 22.5  $\mu$ l 1-methyl imidazole  
and 52.5 mg EDC are added to 625  $\mu$ l water, and the reaction is allowed to proceed for  
one hour. The resin is combined with the ligand, and the reaction is allowed to proceed  
overnight.

20

#### EXAMPLES 101-105

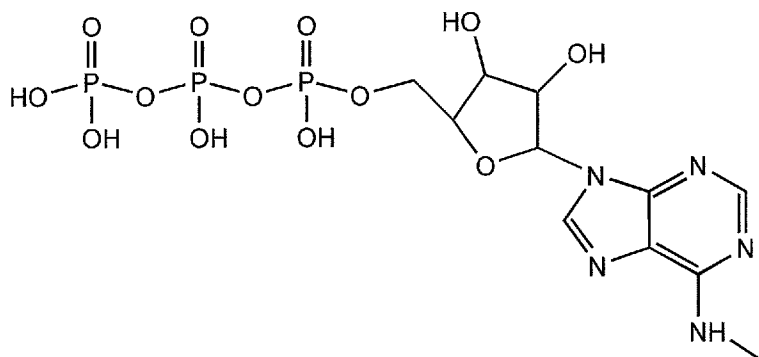
The alkyl-linked nucleotide non-homogeneous solid supports of Examples 101-  
105 are made in three steps: the adenosine derivative is prepared; phosphorylated, and  
then attached to the resin-linker arm combination. For each of examples 99-103, the  
25 adenosine derivatives are phosphorylated as follows. The adenosine derivatives are  
dissolved in trimethyl phosphate (5-12 mL) and cooled to about 0°C. After stirring for 10  
minutes, phosphorous oxychloride (1.8 equivalents) is added dropwise. The reaction  
mixture is stirred at 0°C for 6 hours. Tributylamine (5.4 equivalents) is added followed  
immediately by 0.5 M tributylammonium pyrophosphate (4 equivalents) and stirring is

5 continued for about 15 additional minutes. The reaction is quenched by the addition of  
0.2 M triethylammonium bicarbonate (40-50 mL). The reaction mixture is then stirred at  
room temperature for 15 hours, and then lyophilized. The crude preparation is then  
purified on a Sephadex™ DEAE-25-ion-exchange resin, and eluted with water following  
a gradient of TEAB buffer (0.05 M to 0.5 M). The appropriate fractions are then  
10 combined.

#### EXAMPLE 101

The following structure represents an ATP analog synthesized using the  
conditions described in Example 101:

15

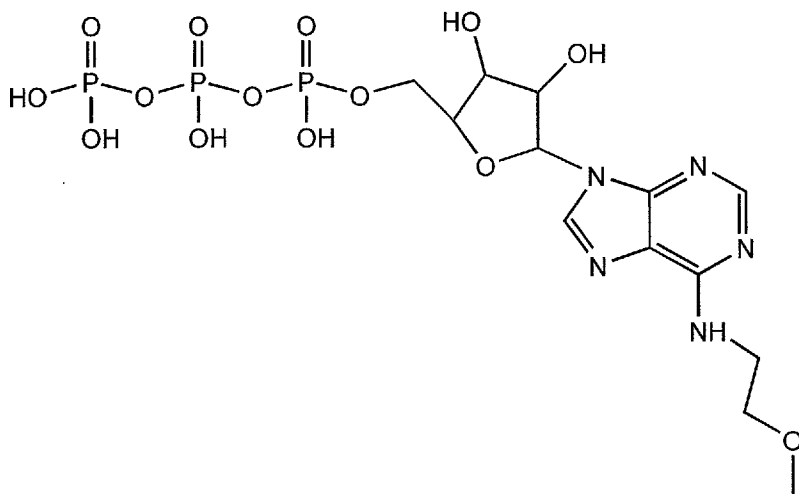


1.016 g 6-chloropurine riboside and 8.82 mL methylamine (33% in ethyl alcohol)  
are combined and heated to 90°C in a sealed reaction vessel for about 22 hours. The  
20 reaction vessel is cooled in an ice bath for about 30 minutes to allow formation of a solid  
precipitate. The precipitate is filtered and washed with ice-cold ethyl alcohol (3 × 25  
mL) and dried to 0.66 g of *N*<sup>6</sup>-methyladenosine in 66% yield.

16 mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added  
to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated  
25 Sepharose™. The reaction is allowed to proceed for about 2 hours. 202 mg ligand, 90 µl  
1-methyl imidazole and 210 mg EDC are added to 1 ml water and allowed to react to  
about one hour. The ligand is then added to the resin and the reaction is allowed to  
proceed overnight.

## 5 EXAMPLE 102

The following structure represents an ATP analog synthesized using the conditions described in Example 102:



10

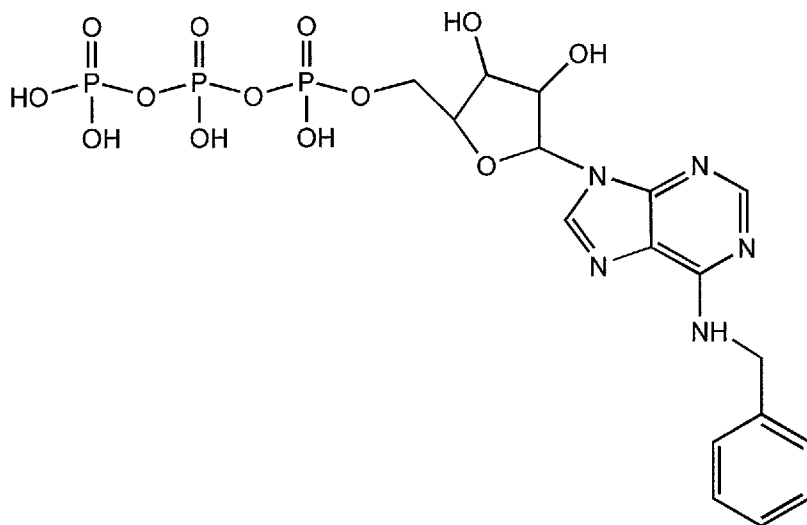
547 mg 6-chloropurine riboside, 0.882 mL 2-methoxyethylamine, and 5.0 mL ethyl alcohol are combined and heated to 90°C in a sealed reaction vessel for about 22 hours. The ligand is then purified by silica gel chromatography using 8:1 methylene chloride:methanol to yield *N*<sup>6</sup>-(2-methoxyethyl)-adenosine (0.61 g, 98% yield).

15 16mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated Sepharose™. The reaction is allowed to proceed for about 2 hours. 154 mg ligand, 90 µl 1-methyl imidazole and 210 mg EDC are added to 1 ml water and allowed to react to about one hour. The ligand is then added to the resin and the reaction is allowed to  
20 proceed overnight.

## EXAMPLE 103

The following structure represents an ATP analog synthesized using the conditions described in Example 103:

25



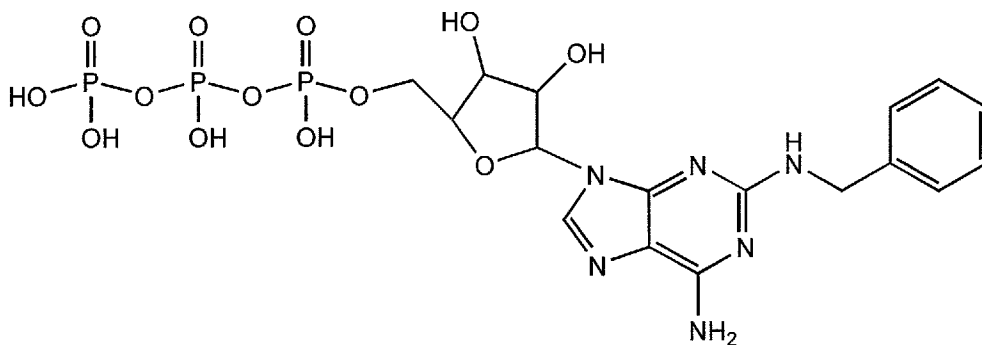
5

473 mg 6-chloropurine riboside, 0.54 mL 2-benzylamine, 0.69 ml triethylamine, and 5.0 mL of ethyl alcohol are combined and heated to 90°C in a sealed reaction vessel for about 18 hours. The reaction vessel is cooled in an ice bath for about 30 minutes to allow formation of a solid precipitate. The precipitate is filtered and washed with ice-cold ethyl alcohol (3 × 25 mL) and dried to yield 0.51 g of *N*<sup>6</sup>-benzyladenosine (86% yield).

16 mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated Sepharose™. The reaction is allowed to proceed for about 2 hours. 160.8 mg ligand, 90 µl 1-methyl imidazole and 210 mg EDC are added to 1 ml water and allowed to react to about one hour. The ligand is then added to the resin and the reaction is allowed to proceed overnight.

## 20 EXAMPLE 104

The following structure represents an ATP analog synthesized using the conditions described in Example 104:



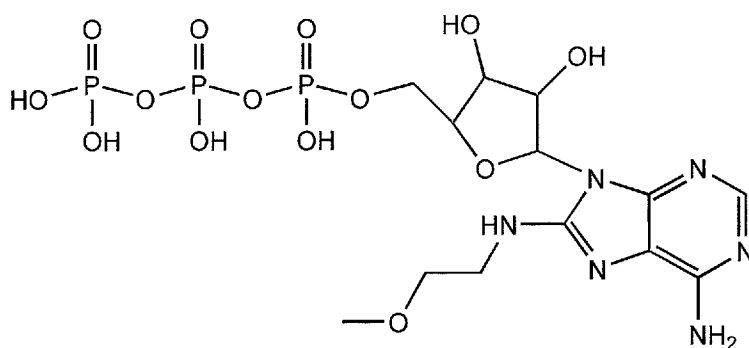
5

400 mg 2-chloroadenosine hemihydrate, 0.42 mL 2-benzylamine, 0.54 mL triethylamine, and 5.0 mL of ethyl alcohol are combined and heated to 90°C in a sealed reaction vessel for about 96 hours. The ligand is then purified by silica gel chromatography using 20:1; methylene chloride:methanol to yield 0.11 g of *N*<sup>2</sup>-benzyladenosine (22%).

16 mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated Sepharose™. The reaction is allowed to proceed for about 2 hours. 120 mg ligand, 67.5 µl 1-methyl imidazole and 157.5 mg EDC are added to 1 mL water and allowed to react to about one hour. The ligand is then added to the resin and the reaction is allowed to proceed overnight.

#### EXAMPLE 105

20 The following structure represents an ATP analog synthesized using the conditions described in Example 105:





5            507 mg 8-bromoadenosine, 0.631 mL 2-methoxyethylamine, and 5.0 mL of ethyl alcohol are combined and heated to 90°C in a sealed reaction vessel for about 168 hours. The ligand is then purified by silica gel chromatography using 4:1, methylene chloride:methanol to yield 0.52 g of *N*<sup>8</sup>-(2-methoxyethyl)-adenosine (99% yield) as a clear, viscous oil.

10           16 mg 1,10-diaminodecane, 16 µl ethanolamine and 125 µl 1,4-dioxane are added to 500 µl coupling buffer and combined with 125 mg cyanogen bromide-activated Sepharose™. The reaction is allowed to proceed for about 2 hours. 157.2 mg ligand, 90 µl 1-methyl imidazole and 210 mg EDC are added to 1 ml water and allowed to react to about one hour. The ligand is then added to the resin and the reaction is allowed to  
15   proceed overnight.

             While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that  
20   various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

             Various publications, patent applications and patents are cited herein, the disclosures of which are incorporated by reference in their entireties.

25

## ALKYL-LINKED NUCLEOTIDE COMPOSITIONS

## ABSTRACT OF THE DISCLOSURE

10           Alkyl-linked nucleotide non-homogeneous solid supports and nucleotide affinity  
media comprising an alkyl-linked nucleotide are provided. The linker is generally a  
hydrophobic linker that can be a 3, 4, 5, 6, 7, 8, 9, 10, or a longer carbon chain. Also  
included in the invention are methods for synthesis of an alkyl-linked nucleotide,  
nucleotide affinity media and methods of use thereof for affinity chromatography and  
15   screening methods.